Hemanext®

Key Scientific Abstracts and Publications

2018-2021

MKTG-0041-US Rev A01

AABB

Abstracts for 2021

OA4-AM21-28

Ultra-High Throughput Metabolomics of a 96-well plate RBC storage platform to screen novel storage solutions

Travis Nemkov[1,2], Maria Nikulina[3], Tatsuro Yoshida[3] and Angelo D'Alessandro[2,4]

(1)University of Colorado–Anschutz Medical Campus, Aurora, CO, (2)Omix Technologies, Aurora, CO, (3)Hemanext Inc., Lexington, MA, (4)University of Colorado–Anschutz, Aurora, CO

Background/Case Studies

Recent investigations using omics technologies have revealed numerous candidate metabolic pathways for intervention to mitigate storage lesion effects associated with RBC storage. However, the iterative process of serially screening variables to improve RBC additive solutions (AS) has hampered development and prohibited implementation of novel AS in the last 2 decades. Therefore, adapting a high throughput screening (HTS) approach commonly used in drug discovery could hold the potential to enhance the efficiency of AS development.

To cater a HTS approach towards RBC storage, we developed a 96 well plate (96WP) storage screening system that is capable of functionally recapitulating storage trends that occur in standard PVC bags. This storage system was validated by high throughput mass spectrometry to compare the metabolome of RBC stored in PVC bags to those stored in 96WP, under both conventional (C) and hypoxic/hypocapnic conditions (H/H). 958

Study Design/Methods

We pooled 2 units of AS3 LR-RBC together (6 replicate pools), after which the pools were split into two bags, and one was processed for H/H storage. Each of the resulting bags of blood (C and H/H) were then plated into 6 separate wells on their own respective 2ml 96-well plates that contained 1 strip (1cm2) of PVC in each well to mimic exposure to a conventional storage bag. The H/H plate was processed in an anaerobic glovebox and stored in an O2 barrier bag with O2/CO2 sorbent. RBC storage homogenate was sampled weekly and stored at -80°C until all samples were collected and analyzed by an ultra-high throughput metabolomics method using a Vanquish UHPLC coupled to a Q Exactive mass spectrometer. 706

Results/Findings

Previously established metabolic traits in anaerobically stored RBC were observed using this 96WP platform, and these results mirrored those from RBC stored in conventional PVC bags. Specifically, 96WP H/H RBC showed higher consumption of glucose at day 42 (H/H vs C fold change, paired T-test p-value): (0.85, 0.0003) and increased production of lactate (1.25, 0.005) compared to 96WP C RBC. Notably, these values were similar when comparing 96WP to PVC bag samples for glucose (0.84, 0.007) and lactate (1.2, 0.0006). In addition, the end-of-storage level of the oxidative stress marker hypoxanthine was lower in the H/H 96WP (0.51, 0.001) and PVC bag (0.70, 0.008) RBC relative to C. 687

Conclusions

Metabolic profiles in C and H/H RBC were comparable between the 96WP and standard PVC storage platforms, and accurately recapitulated known metabolic trends associated with the storage lesion. Considering the multiplexable utility of this platform that enables processing of hundreds of samples in parallel, with streamlined metabolomic readouts using ultra-high throughput mass spectrometry, this platform is suitable to screen multiple candidates for additive solutions to improve the quality of stored RBC. 510

	C		H/H	
	96WP	PVC	96WP	PVC
Glucose	44.8 ± 2.1	43.9 ± 0.2	37.8 ± 2.9	37.3 ± 2.6
Lactate	541.7 ± 51.9	552.5 ± 44.2	680.4 ± 15.6	685.0 ± 40.3
Hypoxanthine	8.8 ± 0.8	10.3 ± 0.7	5.5 ± 1.4	6.4 ± 1.7

Table 1: Select metabolites from dataset - Average ± standard deviation for glucose, lactate, and hypoxanthine levels (in normalized arbitrary units) at Day 42.

P-NI-4

A deep 96-well plate RBC storage platform for a high-throughput screening of novel storage solutions

Maria Nikulina[1], Travis Nemkov[2], Angelo D'Alessandro[2], Tatsuro Yoshida[1]

(1)Hemanext Inc., Lexington, MA (2)Omix Technologies, Aurora, CO

Background/Case Studies

Over the past 40 years, development of RBC additive solutions took place by modifying existing solution serially, which is a highly time consuming and labor-intensive process and no new additive solutions have been adopted by blood banks since AS-3 in 2000s.

To broaden the scope of additive formulations and accelerate the pace of investigations, we developed a high-throughput RBC storage process.

Specifically, this study was undertaken to compare standard PVC bag storage vs. the use of 2ml 96 well plate for RBC storage in terms of weekly hemolysis and ATP. As DEHP in PVC bags has been shown to help reduce hemolysis, this study also focused on investigating the effects of including PVC strips. The study was conducted using both the conventional method (C) and hypoxic/hypocapnic methods (H/H). 803

Study Design/Methods

We procured a total of 8 units of AS3 LR-RBC within 24 hours of collection. Four pairs of compatible units were pooled together, after which the pools were split into two bags, one of which was processed for H/H storage and the other was conventionally stored. Each of the 8 resulting bags of blood was then plated into their own 2ml 96-well plate, yielding a total of 8 plates, 4 H/H and 4 C. A third of wells in each plate had no PVC strips, a third had 1 strip and a third had 2 strips added. The size of the strip (1cm2) was cut proportional to the amount of surface area of PVC that is exposed to a 2ml volume of blood in a blood storage bag. The 4 H/H plates were processed in an anaerobic glovebox and stored in O2 barrier bags with an oxygen sorbent. Supernatant hemoglobin and ATP were measured weekly using standard methods. 834

Results/Findings

There was a significant difference between the hemolysis for no strips vs. 1 and 2 strips ($p < 0.05$, paired T-test) for samples in C and a significant difference for the no strips vs 2 strips samples in H/H. For ATP there was a significant difference in ATP amount between the samples with no strip vs 1 strip in C and no strip vs 2 strips in H/H.

Both ATP and hemolysis values are comparable to the typical values observed for AS-3 LR-RBC 6-week storage in standard storage bags under C and H/H. The final hemolysis values were below 0.8%. 714

Conclusions

Observed hemolysis and ATP profiles were comparable in RBC storage using deep 96-well platform compared to standard volume PVC-bag, both in C and H/H. Including PVC strip in a polypropylene well resulted in a small but significant reduction in hemolysis. Combined with the metabolomics workflow, the deep 96-well plate storage platform is suitable for high-throughput storage studies examining the composition of novel additive solutions. 446

	C			H/H		
	0 strip	1 strip	2 strips	0 strip	1 strip	2 strips
Hemolysis (%)	0.35	0.26	0.27	0.42	0.38	0.32
ATP (µmol/gHb)	2.23	2.54	2.47	2.88	2.80	2.49

Table 1: Average hemolysis and ATP levels at Day 42

P-NI-15

Hematocrit to viscosity ratio an index of oxygen transport efficiency of red blood cells is increased by hypoxic storage: implications for blood transfusion

Samuel Sowemimo-Coker[1], Marion Ceniza[1], Marie L Charles-Delva[1], Laurel A Omert[1]

(1) Hemanext, Lexington, MA

Background/Case Studies

The hematocrit to viscosity ratio (HVR) is an index of red cell oxygen transport effectiveness that varies with shear stress and hematocrits. It balances the benefits of improved oxygen capacity to viscosity-mediated impairment of microvascular flow. High viscosity will impede microcirculation while low hematocrit will fail to address anemia. Therefore, the HVR provides a metric that considers the impact of each variable on oxygenation of the microvascular beds. High HVR results in an improved oxygen delivery while low HVR results in an impairment of oxygen delivery efficiency. High HVR has been shown to correlate with improvement in recurring leg ulcers and microvascular oxygenation in sickle cell disease. The main objective of the present study was to measure the HVR of red blood cells (RBCs) stored under hypoxic and conventional storage conditions.

Study Design/Methods

Six units of 1-day old O Negative leukocyte reduced RBCs in AS3 were obtained from Rhode Island Blood Center. The units were pooled in pairs and divided into 300±10mL each for storage under conventional condition (A) and hypoxic storage condition (B). Unit B was deoxygenated at 22±2°C for 3 hours so that the percent oxygen saturation of the hemoglobin of the RBCs was reduced to less than 20%. The deoxygenated RBCs were stored in an oxygen impermeable storage bag for 42 days at 4°C. On day 42 of storage, the units were resuspended in AB universal plasma at different hematocrits (10%, 20%, 30%, 40% and 50%). The viscosities of the different samples were measured at 40 different shear rates at 37±1°C with a cone plate viscometer. Differences between hypoxic and control were analyzed with Student “t” test for paired data.

Results/Findings

The results are summarized in the Table as the means±standard deviations of 3 independent pools. The HVRs of hypoxic RBCs were higher than control *p<0.05. At 50% hematocrit, HVR decreased at low shear rates compared to 30%, **p<0.05

Shear rate (1/sec) at different hematocrits	HVR	HVRs at different storage condition on day 42	
30% Hematocrit	Day 0	Control	Hypoxic
7	8.5±0.0	5.8±0.8	7.1±1.2*
10	8.6±0.8	7.3±0.3	8.4±0.78*
20	9.6±0.0	7.9±0.4	8.8±0.4*
50	9.9±0.1	8.7±0.3	9.4±0.2
100	10.6±0.2	9.6±0.2	10.1±0.2
130	11.0±0.1	9.9±0.2	10.7±0.1*
50% Hematocrit			
7**	5.1±0.1	4.1±0.2	4.6±0.5
10**	6.4±0.3	4.8±0.2	5.5±0.3*
20**	7.5±0.2	6.0±0.3	6.6±0.5
50	9.6±0.1	7.8±0.3	8.7±0.5*
100	11.1±0.3	9.5±0.3	10.4±0.6*
130	11.5±0.4	10.1±0.4	11.0±0.6*

Conclusions

These preliminary results show that hypoxic RBCs have higher HVRs than conventionally stored RBCs and suggest better oxygen transport efficiency. RBCs with high HVR may benefit patients with impaired microvascular blood flow who receive chronic transfusion such as those with sickle cell disease.

AABB

Key Publication and Abstracts for 2018-2020

P-BB-22

Estimating Oxidative Stress Burden of Multiple Blood Transfusions in Trauma Patients

Sarah E. DePew[1], Nirupama J. Singh[2], Marisa B. Marques[2], Rakesh Patel[3], Laurel A Omert[4], Andrew Dunham[4] and Tatsuro Yoshida[4]

(1)The University of Alabama at Birmingham, Birmingham, AL (2)Department of Pathology, University of Alabama at Birmingham, Birmingham, AL (3)University of Alabama at Birmingham, Birmingham, AL (4)Hemanext, Lexington, MA

Background/Case Studies

Bleeding trauma patients require transfusion (Tx) of multiple units of packed red blood cells (PRBCs) or whole blood (WB) for resuscitation. Transfusing older blood is associated with poorer outcomes due to “storage lesions” characterized by oxidative damage to RBCs. (Yoshida Blood Transfus. 2019). Studies demonstrated that oxygen concentration (O2) in PRBC units varies widely due to donor factors (ibid Yoshida 2017) as well as oxygen ingress into the bag during storage. Similar observations are reported with WB (Joo-Yeun; J Trauma & ACS 2020). Trauma patients requiring multiple units are therefore at higher risk from cumulative exposure to oxidatively damaged red cells.

Study Design/Methods

This study aims to determine the O2 content of PRBC and WB units at time of Tx, and estimate the oxidative stress burden a trauma patient receiving multiple units might sustain. Hb O2 saturation levels (%SO2) of 135 PRBCs and 100 WB in trauma coolers were measured non-invasively (Moor VMS-OXY, UK). SO2 of Day 0 (D0) and Day of transfusion (DTx) were calculated from age of units on Day of measurement (Dm) using a numerical simulation model (unpublished data, McMahon et al, Irish Blood Tx Service). To estimate cumulative O2 exposure in 8 patients, an O2 exposure index (OEI), defined as AUC of SO2 vs. day-in-storage curve (unit=SO2 *D), was calculated for each unit.

Results/Findings

Mean age of 135 PRBC at DTx was 13.8 ±3.6D. Estimated SO2 (eSO2) at D0 ranged from 28 – 92% (Median,M=61), calculated from SO2 at Dm which varied from 42 - >95%. Dtx eSO2 ranged from 28 – 100% (M 72). OEI ranged from 29-1204U (mean 625 ± 242U). OEI burden in the majority of transfused PRBC was 418 to 802U.

Mean age of the 100 WB at DTx was 11.6 ±3.2D. eSO2 at D0 ranged from 4- 69% (M 33), calculated from SO2 at Dm which varied from 16 - 73%. DTx eSO2 ranged from > 21 – 79%(M 45). OEI was 0-815U (M 273 ± 178). OEI burden in the majority of transfused WB was 151 to 335U.

OEI burden in 8 patients who received multiple units of PRBC (Avg 6.5 units, 14D, eSO2=71%) and WB (4.4U, 10D, 46%) was estimated. Mean total OEI burden per patient was 5100U (range 2400-9600). Average OEI was 667U and 209U for PRBC and WB units respectively.

Conclusions

The estimated OEI burden of PRBC and WB varied widely at time of Tx and, despite similar mean age of units, was lower in WB. A multiple transfused trauma patient can be exposed to OEI burdens of at least 2000U. Hypoxic storage of RBCs (pre-storage O2 reduction and an oxygen impermeable storage bag) can minimize the O2 burden of stored PRBCs and eliminate variability in Tx. A randomized controlled trial comparing low and high OEI burdens would shed light on conflicting trial results regarding age of blood.

P-BB-32

Hypoxic Storage of Donor Red Cells Improves Deformability after Exposure to Plasma from Adults with Sickle Cell Disease during Vaso-Occlusive Crisis

Matthew S. Karafin[1], Sergey S. Shevkoplyas[2], Marie L. Charles-Delva[3], Marion Ceniza[3], Joshua Field[4] and Tatsuro Yoshida[3]
(1)Versiti, Milwaukee, WI (2)University of Houston, Houston, TX (3)Hemanext, Lexington, MA (4)Medical College of Wisconsin, Milwaukee, MN

Background/Case Studies

It is well established that red cell (RBC) transfusions are clinically beneficial for patients with sickle cell disease (SCD), but previous ex vivo studies suggest that the inflamed plasma (PL) from patients with SCD during vaso-occlusive pain crisis (VOC) may damage these RBCs, diminishing their potential efficacy. The hypoxic storage of RBCs may improve transfusion efficacy by minimizing the storage lesion, but the degree to which storage conditions impact RBC function after exposure to SCD PL has not been previously investigated.

Study Design/Methods

We obtained PL from 18 adults with SCD and froze aliquots at -80°C; half were at their baseline (BL) level of health, and half were hospitalized with VOC. Eight ABORh-compatible units of leukoreduced RBCs suspended in AS-3, were split equally and stored under conventional (CRBC) and hypoxic (HRBC) conditions for 42 days. The RBCs were mixed with PL (1:1) and incubated overnight at 37°C. Each RBC unit/PL sample pair was tested for hemolysis and the ability to perfuse an artificial capillary network. The network perfusion rate was normalized by an internal standard to increase comparability between samples. These outcome measures were compared statistically by patient group and storage type.

Results/Findings

Capillary network perfusion was modestly but significantly higher for HRBC than for CRBC when incubated with PL from both patient groups (BL: 7.3% increase, $p<0.0001$, VOC: 8.9% increase, $p<0.0001$); perfusion was worse after incubation in VOC than in BL PL (CRBC: 8.3% decrease, $p=0.03$, HRBC: 7.0% decrease, $p=0.06$) (Table 1). Interestingly, the perfusion rate varied substantially among different RBC units, independently of storage conditions or patient group (range: 0.43-0.58). Lastly, incubation with VOC PL increased hemolysis significantly only for CRBC (11.0% increase, $p=0.03$).

Conclusions

In SCD, clinical state impacts the mechanical properties and viability of donor RBC, and this impact can be mitigated, in part, by modifying the storage method. Other important factors may also drive unit quality, as RBC variability seemed to occur regardless of the PL-type or storage method. Future prospective studies will be needed to confirm the clinical importance of these ex vivo observations.

Table 1: Mean relative perfusion rate and %hemolysis after 24-hour incubation by patient group and RBC type.

Plasma – RBC storage pair	Hemolysis (standard error)	Relative perfusion (standard error)
Baseline – Conventional	3.92 (0.14)	.48 (0.014)
Baseline – Hypoxic	3.84 (0.14)	.52 (0.014)
Crisis – Conventional	4.35 (0.14)	.44 (0.014)
Crisis – Hypoxic	4.08 (0.14)	.48 (0.014)

P-NI-11

Hypoxic Storage in Novel Non-DEHP Bags Improves Red Blood Cell Quality during 56 Day Storage at 4°C

Samuel Sowemimo-Coker[1], Marion Ceniza[1], Marie L Charles-Delva[1], Laurel A Omert[1]

(1) Hemanext, Lexington, MA

Background/Case Studies

The quality of aerobic, refrigerated stored red blood cells (RBCs) deteriorates progressively during storage, a process collectively called “the RBC storage lesion”, which may negatively impact oxygen delivery and clinical outcome. Oxidative damage of RBC membranes by reactive oxygen species is a major contributor to the storage lesion. Hypoxic storage conditions have been shown to reduce oxidative damage and better maintain the quality of the stored RBCs. Currently, most RBCs are stored aerobically in polyvinyl chloride (PVC) bags plasticized with di(2-ethylhexyl)phthalate (DEHP) plasticizer a potential human carcinogen. In order to reduce oxidative damage to RBCs and prevent DEHP-induced toxicity, non- DEHP hypoxic storage bags (HYPX) were developed. The aim of the present study was to compare the *in vitro* quality of RBCs stored in HYPX and standard PVC DEHP bags for 56 days at 4°C.

Study Design/Methods

Twenty four units of whole blood (500±50mL) were collected into CP2D anticoagulant. Each unit was processed into plasma and a leukocyte-reduced red cell concentrate (LR-RCC) in AS7 supplemented with 2mM guanosine, 1mM N-acetyl cysteine, 1mMTrolox, 0.50mM vitamin C and 40mM bicarbonate. For each test, three units (300-340mL each) of ABO matched LR-RCC were pooled together into a 2-liter non-DEHP bag. Equal aliquots (300mL) from the pool were transferred into DEHP and HYPX bags (Hemanext, Lexington, MA, USA). Several *in vitro* metrics of RBCs were measured before and during 56-day storage at 4±2°C including hemolysis, ATP, 2,3DPG, methemoglobin and gas panel. Differences between HYPX and DEHP bags were analyzed with repeated-measures analysis of variance with Newman-Keuls multiple comparison test.

Results/Findings

The results are summarized in the Table as the means±standard deviations of 8 independent pools of LR-RCC (N=8). The percent oxygen saturation of the hemoglobin in the RBCs (%SO2) was lower in the HYPX than conventional storage resulting in significant reduction in the formation of methemoglobin a marker of oxidative damage.

Hypoxic Storage in Novel Non-DEHP Bags Improves Red Blood Cell Quality during 56 Day Storage at 4°C – continued

In vitro Parameters	Day 0	Type of Storage Bag			*p
Day 21		DEHP	HYPX-A	HYPX-B	
% SO2	48±4	71±6	42±6	42±5	P<0.001
ATP (µmol/g Hb)	3.9±0.3	3.8±0.4	4.2±0.6	4.1±0.6	P<0.01
2,3DPG (µmol/g Hb)	11.4±1.0	3.0±2.4	6.2±5.2	10.8±4.1	p<0.01
Hemolysis (%)	0.08±0.03	0.15±0.06	0.17±0.06	0.18±0.06	p>0.05
Methemoglobin (%)	0.59±0.07	0.79±0.09	0.71±0.13	0.74±0.10	p>0.05
Day 42					
% SO2	48±4	83±8	30±14	31±9	P<0.05
ATP(µmol/g Hb)	3.9±0.3	3.0±0.6	4.2±0.7	4.4±0.9	p<0.001
2,3DPG(µmol/g Hb)	11.4±1.0	2.0±2.5	3.6±4.2	5.7±3.0	p<0.05
Hemolysis (%)	0.08±0.03	0.24±0.06	0.29±0.08	0.33±0.10	P>0.05
Methemoglobin (%)	0.59±0.07	0.96±0.08	0.77±0.15	0.77±0.10	P<0.05
Day 56					
% SO2	48.±4	88±10	25±12	23±12	P<0.001
ATP(µmol/g Hb)	3.9±0.3	2.3±0.5	3.8±1.3	3.8±0.8	P <0.001
2,3DPG(µmol/g Hb)	11.4±1.0	1.3±0.7	2.0±0.9	4.6±2.4	P<0.01
Hemolysis (%)	0.08±0.03	0.30±0.08	0.43±0.14	0.47±0.18	P<0.05
Methemoglobin (%)	0.59±0.07	1.10±0.18	0.79±0.28	0.84±0.26	P<0.05

*DEHP versus Hypoxic storage bags

Conclusions

ATP, 2,3DPG are better maintained in HYPX storage condition than storage in PVC with corresponding reduction in oxidative damage. RBC hemolysis levels in HYPX bags at 56-day storage are below 0.8% thus meeting regulatory requirement. Preliminary data from ongoing studies show similar improvements in RBC quality in AS3 and PAGGSM storage solutions.

P-NI-12

Improved Quality of CP2D/AS-3 Red Blood Cells Processed and Stored for 42 days in the Hemanext Oxygen Reduction System After X-Ray Irradiation at Day 14 or Day 21

Pamela H. Whitley[1], Michael S. Wellington[1], Philip Howard[1], Sherrie L. Sawyer[1], Lorelei Hopersberger[1], Michael Dioguardi[2], William Iselin[2] and Tatsuro Yoshida[2]

(1)American Red Cross, Norfolk, VA, (2)Hemanext, Lexington, MA

Background/Case Studies

Irradiation of red blood cells (RBC) is the standard for preventing transfusion associated graft versus-host disease (TA-GVHD) but has been shown to have a deleterious effect that results in reducing storage time to 28 days post irradiation. Previous studies indicate potential benefits of the Hemanext Oxygen Reduction System (ORS) on the RBC storage lesion after gamma irradiation. In this study, the effects of Day 14 or 21 X-ray irradiation on AS-3 RBC processed with the ORS are compared to Day 14 or 21 X-ray irradiation of conventional AS-3 RBC.

Study Design/Methods

Eight pools of four leukoreduced CP2D/AS-3 (32) units from ABO-matched donors were used. Pools were split into equal aliquots and transferred into bags labelled A, B, C, and D. Units A and C (Control) were placed into 4°C storage within 8 hours after Whole Blood (WB) collection. Units B and D (Test) were processed with the ORS and stored at 4°C within 12 hours after WB collection. A-B and C-D were X-ray irradiated at day 14 and 21 of storage respectively. RBC's were stored for 42 days and sampled weekly for CBC, blood gases, SO2, ATP, 2,3 DPG and hemolysis. Differences between the Control and Test were analyzed with Student t-test with $p<0.05$ as significant.

Results/Findings

The results are summarized in the table. The percent saturation of the hemoglobin in the RBC (%SO2) on Day 0 was 11.9 in the hypoxic Test RBC compared to 56.3 in the Control RBC. Hemolysis for Test (D) with later X-ray irradiation (Day 21) was significantly lower in Day 28 and beyond ($p<0.035$) compared to the Control (C).

In vitro Parameters	Storage conditions Mean ± SD		P value
Day 14 A&B (post x-ray)	Test (Hypoxic)	Control (Conventional)	
ATP (µmol/g Hb, n=8)	5.84±0.89	6.02±0.99	N.S
2,3 DPG (µmol/g Hb, n=5)	20.56±2.00	1.13±0.55	p<0.001
Hemolysis (%, n=8)	0.14±0.05	0.20±0.06	P<0.05
Day 21C&D (post x-ray)	Test	Control	
ATP (µmol/g Hb, n=8)	5.83±0.93	5.59±1.15	N.S
2,3 DPG (µmol/g Hb, n=4)	12.81±1.58	1.41±1.04	p<0.05
Hemolysis (%, n=8)	0.14±0.07	0.21±0.05	p<0.05
Day 42/43 (A&B&C&D)	Test	Control	
ATP (µmol/g Hb, n=16)	3.84±1.18	3.32±0.63	p<0.001
2,3 DPG (µmol/g Hb, n=9)	1.38±1.17	0.57±0.67	p<0.001
Hemolysis (%, n=16)	0.22±0.09	0.34±0.07	p<0.001

N.S.: not significant

Conclusions

RBC processed with the ORS and stored for 42 days shows significantly higher 2,3 DPG, ATP and lower hemolysis over conventional RBC, when irradiated at Day 14 or 21. Additionally, Hemanext ORS reduced the increases in post x-ray hemolysis.

Note: The sample size for the 2,3 DPG data in this abstract has been updated for accuracy and differs from what is reflected in Transfusion vol 60 issue S5.

Transfusion of Anaerobically or Conventionally Stored Blood After Hemorrhagic Shock

Williams, Alexander T.*; Jani, Vivek P.*; Nemkov, Travis†; Lucas, Alfredo*; Yoshida, Tatsuro‡; Dunham, Andrew‡; Alessandro, Angelo D'†; Cabrales, Pedro*

*Department of Bioengineering, University of California, San Diego, La Jolla, California, †Department of Biochemistry and Molecular Genetics, University of Colorado Denver, Aurora, Colorado, ‡Hemanext, Lexington, Massachusetts

Shock: August 30, 2019 - Volume Publish Ahead of Print
doi: 10.1097/SHK.0000000000001386

ABSTRACT

Background

Resuscitation from hemorrhagic shock (HS) by blood transfusion restores oxygen (O2) delivery and provides hemodynamic stability. Current regulations allow red blood cells (RBCs) to be stored and used for up to 42 days. During storage, RBCs undergo many structural and functional changes. These storage lesions have been associated with adverse events and increased mortality after transfusion, increasing the need for improved RBC storage protocols. This study evaluates the efficacy of anaerobically stored RBCs to resuscitate rats from severe HS compared with conventionally stored RBCs.

Methods and Results

Rat RBCs were stored under anaerobic, anaerobic/hypercapnic, or conventional conditions for a period of 3 weeks. Hemorrhage was induced by controlled bleeding, shock was maintained for 30 min, and RBCs were transfused to restore and maintain blood pressure near the hemorrhage level. All storage conditions met current regulatory 24-h posttransfusion recovery requirements. Transfusion of anaerobically stored RBCs required significantly less RBC volume to restore and maintain hemodynamics. Anaerobic or anaerobic/hypercapnic RBCs restored hemodynamics better than conventionally stored RBCs. Resuscitation with conventionally stored RBCs impaired indices of left ventricular cardiac function, increased hypoxic tissue staining and inflammatory markers, and affected organ function compared with anaerobically stored RBCs.

Conclusions

Resuscitation from HS via transfusion of anaerobically stored RBCs recovered cardiac function, restored hemodynamic stability, and improved outcomes.

OA3-ST4-29

Metabolic Predictors of 24h Post-Transfusion Recovery in End of Storage Control and Hypoxic Red Blood Cells

Angelo D'Alessandro[1], Travis Nemkov[1], Davide Stefanoni[1], Michael Dioguardi[2], Neeta Rugg[3], Shawnagay Nestheide[3], William Iselin[2], Andrew Dunham[2], Jose A. Cancelas[3] and Tatsuro Yoshida[2]

(1)University of Colorado Denver, Aurora, CO (2)Hemanext, Lexington, MA (3)Hoxworth Blood Center, Cincinnati, OH

Background/Case Studies

As per FDA requirements, end of storage blood quality can be predicted in part by measurements of hemolysis and 24-hr post-transfusion recovery (PTR24). However, PTR24 studies are logistically and economically demanding. As such, the identification of easier to measure markers of stored blood quality can expedite the development and testing of novel storage strategies, such as storage under oxygen-controlled conditions.

Study Design/Methods

RBCs were stored under normoxic or hypoxic conditions in a pivotal, prospective, randomized, two-arm, crossover trial (n=12 donors with paired normoxic and hypoxic storage) and sampled at Days 0, 21, and 42. RBC and supernatant fractions were analyzed separately using a high-throughput LC-MS based metabolomics platform. End-of-storage day 42 metabolite measurements were compared to PTR24 measurements. Since hypoxic storage greatly affects RBC metabolism as well as oxidative stress, normoxic and hypoxicdata sets were analyzed separately.

Results

The relative levels of 302 metabolites and lipids were monitored at storage days 0, 21, and 42. Analysis of Pearson's correlations between the levels of these compounds at storage day 42 and PTR24 determined an $|R| \geq 0.5$ for 37 compounds in normoxic RBCs (15 with $p \leq 0.05$), and 41 compounds in hypoxic RBCs (19 with $p \leq 0.05$). The top 5 correlating and inversely correlating compounds for each condition are reported along with p-values in the table. For normoxic storage, strong correlations are primarily seen with membrane lipids and associated fatty acids, amino acids, and Pentose Phosphate Pathway intermediates. Hypoxic storage PTR24 values primarily correlate with alternative membrane components, amino acids, and Pentose Phosphate Pathway intermediates.

	Normoxia			Hypoxia	
Compound	**R**	**p-value**	**Compound**	**R**	**p-value**
Decanoic acid	0.81	0.002	Hydoxyhexadecenoylcarnitine	0.81	0.001
Prostaglandin E2	0.69	0.012	Glucose-phosphate	0.72	0.009
Ribose phosphate	0.66	0.020	15:0 Lyso PC	0.69	0.012
Hexadecanoic acid	0.66	0.020	SM(d18:1/24:1)	0.67	0.018
N-Acetylneuraminate	0.57	0.052	Eicosatrienoylethanolamide	0.67	0.018
3-Methyldioxyindole	-0.68	0.015	PE(16:0/20:4)	-0.59	0.046
L-phenylalanine	-0.69	0.012	PC(10:0/20:4)	-0.60	0.039
PE(18:0/20:4)	-0.73	0.585	UMP	-0.67	0.017
L-leucine	-0.74	0.006	L-glutamate	-0.67	0.017
Fe(III)dicitrate	-0.77	0.003	Hexanoic acide	-0.68	0.015

Conclusions

Although normoxic and hypoxic stored RBCs show differing metabolite correlates with PTR24, significant associations tend to occur within similar overall pathways. Comparing the PTR24-metabolite correlations between normoxic and hypoxic RBC storage reveals that intermediates of oxidative stress management and membrane homeostasis are potential predictors of post-transfusion recovery.

P-BB-50

Additive Solutions Differentially Influence the Effects of Hypoxic Storage on Red Blood Cell In Vitro Quality

Joanne C. G. Tan[1,2], Htet Htet Aung[1], Yeojoon Cha[1], Denese C. Marks[1,2], Neil Beckman[3], Andrew Dunham[3]

(1)Clinical Services and Research, Australian Red Cross Blood Service, Alexandria, Australia (2)Sydney Medical School, University of Sydney, Camperdown, Australia (3)Hemanext, Lexington, MA, USA

Background

Hypoxic storage of red blood cells (RBC), achieved by depletion of oxygen (O2) and carbon dioxide (CO2), is reported to influence RBC metabolism and maintain 2,3-DPG. Differences in blood processing methods and RBC additive solutions may also impact RBC metabolism during storage. This study assessed the impact of hypoxic and conventional normoxic storage conditions using three RBC additive solutions, SAG-M, AS-3 or PAGGSM.

Study Design/Methods

RBC components were prepared from whole blood held overnight (<18 hours) using top-and-bottom separation and resuspended in three different additive solutions; SAG-M, AS-3 or PAGGSM. Pairs of RBC in each additive solution were then pooled and split; one of each pair was processed with the Hemanext® RBC Processing System and the other stored in conventional storage bags. RBC in each study arm (n=8 replicates) were tested on day 2, 7, 14, 21, 28, 35 and 42 for biochemical characteristics and biomodulatory capacity. Data were analyzed by two-way repeated measures ANOVA adjusted for multiple comparisons; $p<0.05$ was considered significant.

Results

Hypoxic storage reduced the percent oxygen saturation (SO2) of the hemoglobin in the RBC to <20%, with partial pressure of oxygen (pO2) at 37°C maintained at <20 mmHg throughout storage in all additives. RBC in PAGGSM consumed significantly more glucose ($p=0.0032$) and RBC in all additives produced more lactate ($p<0.0001$). Only hypoxic red cells stored in AS-3 had significantly higher ATP ($p=0.0132$). RBC stored under hypoxic conditions in all additive solutions maintained significantly higher 2,3-DPG concentrations than conventionally stored RBC ($p<0.0001$ for all additives), and 2,3-DPG was over 10-fold higher on day 7, 14 and 21 in hypoxic RBC stored in SAG-M and PAGGSM. Hemolysis was slightly higher in RBC stored hypoxically in AS-3, PAGGSM and SAG-M, although not statistically significant ($p=0.2822$, 0.5977 and 0.1555 respectively) and still well below the Council of Europe and AABB limits of <0.8% and <1.0% respectively. Hypoxic storage of RBC in SAG-M also led to significantly higher potassium release ($p< 0.0001$), as well as increased binding of anti-CD47 2D3 antibody, suggesting a conformational change associated with increased red cell clearance. Supernatants from RBC stored hypoxically or conventionally did not activate human umbilical vein endothelial cells, with no significant differences in secretion of IL-8, IL-6, RANTES or sCD62P, or expression of endothelial cell surface activation markers E-selectin and V-CAM.

Conclusions

Hypoxic storage of RBC better maintains 2,3-DPG compared to conventional storage. Based on the present in vitro data on RBC quality, hypoxic storage is suitable for use with AS3 and PAGGSM and not with SAG-M.

P-BB-61

In Vitro RBC Deformability is a Predictor of Long-Term Stored RBC PTR24 In Vivo while ATP is a Predictor of RBC PTR24 in Hypoxic Stored RBC

Tatsuro Yoshida[1], Neeta Rugg[2], Pamela H. Whitley[3], Michael Dioguardi[1], Scott Brooks[4], William Iselin[1], Philip Spinella[5], Fay West[6], Matthew S. Karafin[7] and Jose Cancelas[2]

(1)Hemanext, Lexington, MA (2)Hoxworth Blood Center, Cincinnati, OH (3)American Red Cross, Norfolk, VA (4)BloodCenter of Wisconsin, Milwaukee, WI (5)St. Louis Children's Hospital, St. Louis, MO (6)American Red Cross Blood Services, Farmington, CT (7)Versiti, Milwaukee, WI

Background/Case Studies

Autologous 24-hr post-transfusion recovery (PTR24) measured in healthy volunteers may not translate to the efficacy of transfused RBCs in recipients. However, with hemolysis, they are metrics mandated by the FDA for evaluating stored RBC. Identification of biomarkers for PTR permits effective pre-screening before conducting human *in vivo* study.

Study Design/Methods

PTR24 data set* (n=14 pairs, normoxic (N) and hypoxic (H) storage, plus 10 unpaired units) from a pivotal, prospective, randomized, two-arm, crossover, two-center trial was analyzed for correlations with in vitro metrics (excluding metabolomics data). Since H storage greatly affects RBC metabolism as well as oxidative stress, N and H storage data sets were analyzed separately. Parameters measured at day (D) 42 included ATP, 2,3-DPG (DPG), hemolysis, MCV, MCHC, MCH, RDW, hematocrit, SO2,blood gas, pH, K+, Na+, glucose, lactate, morphology, microparticles, RBC deformability in a microfluidic system mimicking microvascular networks, as well as rejuvenated ATP and DPG levels. Additionally, SO2, pH, lactate, ATP, DPG at D21, and SO2, pCO2, pH, ATP, and DPG at D0 were examined. All except microparticles and D42 SO2 passed Kolmogorov-Smirnov Test for normality.

Results/Findings

Pearson's correlation coefficients for PTR24 (double label) vs. other parameters were examined and significant ($p<0.05$) R are shown in the table. Using only variables in bold, multiple linear regression for PTR24 yielded R2 of 0.32 and 0.45 for N and H RBCs, respectively. By adding other physiologically relevant variables which did not meet the significance level ($p>0.05$), better fits were achieved with stepwise backward regression analysis ($a>0.1$ removed; N, R2=0.54 with deformability, ATP, pCO2, and glucose; H, R2= 0.75 with ATP, pH, DPG, hemolysis, D21 DPG, pH and D0 ATP).

N: Control (N=21) H: Hypoxic (N=19)	Pearson correlation coefficient	PTR24 N(%)84.8±6.	PTR24 H(%) 89.3±4.5
RBC deformability (nL/s)	R	**0.475**	0.024
N: 0.19±0.02; H: 0.19±0.03	p-value	**0.030**	0.922
ATP (µmol/gHb)	R	**0.442**	**0.522**
N: 3.31±0.73; H: 3.54±0.83	p-value	**0.045**	**0.023**
ATP Rejuvenation (µmol/gHb)	R	**0.461**	0.302
N: 8.16±1.14; H: 8.15±0.79	p-value	**0.035**	0.209
DPG Rejuvenation (µmol/gHb)	R	-0.271	**-0.461**
N: 11.6±3.8; H: 11.9±3.5	p-value	0.235	**0.048**
D0 ATP (µmol/gHb)	R	0.280	**0.569**
N: 3.94±0.58; H: 3.85±0.41	p-value	0.219	**0.014**

**Radio-labeling for 14 subjects occurred without prior RDRC approval; site did not meet GMP standards.*

Conclusions

RBC ATP as well as rejuvenations correlated with PTR24. Correlation differed between N and H RBCs. RBC deformability correlated only with N-stored RBC, while reduced oxidative damage in H-stored RBC removed its dependency. H-stored RBC showed stronger correlations with ATP and other metabolic parameters.

P-BB-62

Oxygen Saturation of Collected RBC Products is Donor Dependent

Tatsuro Yoshida[1], Pamela H. Whitley[2], Neeta Rugg[3], Michael Dioguardi[1], Michael S. Wellington[2], Scott Brooks[4], William Iselin[1], Fay West[5], Matthew S. Karafin[6] and Jose Cancelas[3]

(1)Hemanext, Lexington, MA (2)American Red Cross, Norfolk, VA (3)Hoxworth Blood Center, Cincinnati, OH (4)BloodCenter of Wisconsin, Milwaukee, WI (5)American Red Cross Blood Services, Farmington, CT (6)Versiti, Milwaukee, WI

Background/Case Studies

Oxygen (O2) is a critical element affecting oxidative damage and metabolic homeostasis of RBCs during long-term storage. O2content of RBC unit is represented by SO2 (fractional hemoglobin O2 saturation) which varies unexpectedly in freshly prepared RBC units, while factors affecting observed wide distribution are unknown. Initial SO2 affects cumulative O2 exposure and resulting accumulation of oxidative damage as O2 content increase during storage

Study Design/Methods

Data for two consecutive donations (84±21 days apart), collected for a pivotal, prospective, randomized, two-arm, crossover, three-center trial, were tabulated (88 pairs). SO2, CBC, blood gas, lactate, glucose, ATP, 2,3-DPG, morphology, volume, total hemoglobin mass/unit as well as donor's weight, BMI, blood pressure and pulse rate were analyzed for correlations between two donations. Additionally, correlations between difference in the same parameter for two donations were examined. Pearson's correlation coefficients (R) are calculated for each pair ($p < 0.001$ for all reported R).

Results/Findings

SO2 showed moderate correlation between two donations (R=0.56) with a slight decrease in the second donation (0.58±0.15 vs. 0.54±0.15, $p < 0.03$). Hemoglobin mass/unit showed high correlation between two donations (R=0.86). Very high correlations ($R > 0.9$) were observed for MCV and WBC (pre-leukreduction), while other CBC parameters (MCH, MCHC, RDW, tHb, PLT (pre-filter) showed high correlations ($0.7< R < 0.9$). ATP levels correlated moderately (R=0.75) while 2,3-DPG showed lower correlation (R=0.49). pCO2 and pH showed moderate correlations (R=0.58, and 0.61 respectively). Positive change in SO2 correlated with negative change in pCO2(R= 0.6). Very high correlations were observed with parameters relating to long-term status of the donors, such as MCV, WBC count, tHb and other CBC parameters. SO2 showed only moderate correlation, suggesting contributions from factors other than donor genetics.

Conclusions

High correlations were observed between two blood donations 12±3 weeks apart for parameters likely associated with donor's phenotype. Oxygen content of RBC was affected in part by the short-term donor history and contributed to the unexpectedly wide distribution in O2 content of manufactured RBC units..

P-NE-3

Hypoxic Storage Improves Viscoelastic Properties and Reduces Methemoglobin Formation in Gamma Irradiated Red Blood Cells

Sowemimo-Coker SO[1], Ceniza M[1], Delva-Charles ML[1], Dunham A[1]

(1)Hemanext, Lexington, MA, USA

Background

Gamma irradiation of blood products is currently the standard method for preventing transfusion associated graft versus-host disease (TA-GVHD). Gamma irradiation prevents proliferation of the white blood cells that cause TA-GVHD by damaging the DNA either directly by energy deposition or indirectly by generating reactive oxygen species from the water and oxygen content, and these radicals also damage the red blood cells (RBCs). Reducing the available oxygen in the RBCs prior to gamma irradiation and during refrigerated storage for 42 days may reduce the damaging effects of gamma irradiation on the cells. Therefore, the main objective of the present study was to compare the quality of gamma irradiated RBCs stored under conventional aerobic and hypoxic conditions.

Study Design

Two units (300-340mL each) of 1day old leukocyte reduced red cell concentrates (LR-RCC) in AS3 were obtained from Rhode Island blood Center (RIBC). The two units were pooled together, divided into 300mL aliquot A and B. Unit A was transferred into a conventional storage bag A and while unit B processed and stored in Hemanext oxygen reduction and storage system (Hemanext, Lexington, MA) such that the percent oxygen saturation of the hemoglobin in the RBCs (%SO2) was less than 20%. The hypoxic RBCs were transferred into oxygen impermeable storage bag. Both units A and B were stored at 1-6°C for 42 days. The units were gamma irradiated at 25Gy (Caesium 137 Compagnie ORIS Industrie, France) on either day 7 or 14 of storage and then stored until expiry. The following in vitro metrics of RBCs were measured before, after gamma irradiation and during storage: hemolysis, gas panel, extracellular potassium, glucose, lactate, methemoglobin, ATP, 2, 3DPG, metabolomics (hypoxanthine, lipid peroxidation products, antioxidant status etc.), hemolysis and viscoelasticity at 40 different shear rates (Cone plate viscometer, Ametek Brookfield, MA) in AB plasma at 40% hematocrit. Differences between hypoxic and control RBCs were analyzed with Student “t” test statistic for paired data.

Results

The %SO2 in the hypoxic RBCs on day 1 was 10.6±4 and 17.9±8.3% on day 42 compared to 48.8±11.82 and 89.5±4.8% for the control (N=8). Salient results on day 28 after gamma irradiation on day 14 are summarized on table 1. Similar

In vitro Parameters on day 42	Storage conditions		P value
	Hypoxic	Conventional	
Methemoglobin (%)	0.78±0.12	1.04±0.18	P<0.01
Viscosity (cP) at low shear rate 20 1/s	9.86±1.20	12.04±2.25	P<0.01
Viscosity (cP) at high shear rate 100 1/s	6.49±0.35	7.10±0.73	P<0.01
ATP (µmol/g Hb)	2.96±0.83	2.51±0.55	P<0.01
2,3DPG (µmol/g Hb)	1.01±0.47	0.66±0.26	P<0.01
Hemolysis (%)	0.51±0.16	0.57±0.13*	p>0.05

results were obtained when samples were irradiated on day 7.

**6 out of 8 samples were lower than control*

Conclusions

These results show that the overall quality of gamma irradiated hypoxic RBCs in AS3 is better than conventionally stored cells. Additional studies are ongoing to extend the timing of gamma irradiation to 21 and 35 days during storage.

P-NE-5

Effects of Hypoxic Red Blood Cells on Sickling Kinetics of Red Blood Cells from Patients with Sickle Cell Disease

Sowemimo-Coker SO[1], Kuypers F[2], Larkin S[2], Tung G[2], Dunham A[1]

(1)Hemanext, Lexington, MA, USA (2)Children's Hospital of Oakland Research Institute, Oakland, CA

Background

Transfusion therapy with red blood cell (RBC) units is a well-established treatment of patients with sickle cell disease (SCD) to prevent or reduce the severity of the pathological sequalae of the disease. The quality of aerobic, refrigerated stored RBCs deteriorates progressively in time, a process collectively called "the RBC storage lesion", which may negatively impact the clinical outcome after transfusion. Oxidative damage of RBC membranes by reactive oxygen species is a major contributor to the storage lesion. Hemanext hypoxic RBCs (HY) are stored in an oxygen poor environment with partial pressure of oxygen (PO2) of approximately 8mmHg. This hypoxic storage condition has been shown to reduce oxidative damage and better maintain the quality of the stored RBCs in time. Whereas transfusion of these HY RBCs may be beneficial to patients with SCD, deoxygenation of sickle RBC may lead to polymerization of sickle hemoglobin resulting in the typical sickled shape of the sickle red blood cell and down-stream problems in the vasculature. The aim of the present study was to determine whether HY RBCs mixed with SCD RBC under conditions that mimic transfusion would lead to the increase sickled RBC in the mixed population.

Study Design

Two units (300-340mL each) of 1day old leukocyte reduced RBC concentrates in AS3 obtained from Rhode Island blood Center were pooled and divided into 300mL units. One unit (A) was transferred into a conventional storage bag, and the other unit (HY) was processed in the Hemanext oxygen reduction system (Hemanext, Lexington, MA) to PO2 of about 8mmHg, and transferred into an oxygen impermeable storage bag. Both units were stored at 1-6°C until use before expiry. Blood samples from sickle cell patients were collected into sealed EDTA vacutainers. A peristaltic pump was used to mix RBC from either unit with SS-RBCs without exposure to ambient air, to mimic transfusion conditions. After exposure to each other in a 1/1 (v/v) ratio for 1 or 25 minutes, the mixture was added to a glutaraldehyde/formaldehyde solution and the now morphologically fixed cells were analyzed by flowcytometric image analysis (AMNIS) to define the percentage of abnormal shaped RBC in the population.

Results

Most cells in unit A or HY showed a normal morphology. The sickle cell samples contained a significant fraction of abnormally shaped RBC, mainly with the typical shape of an irreversible sickled cell (ISC). Based on the hematocrit and the relative abundance of unit A, HY or SS in the mixture, the expected percentage of abnormal RBC was calculated, and compared to the actual percentage observed. Table 1 shows the results of SS blood mixed with either RBC unit after exposure to each other for 1 or 25 minutes. The expected number of abnormally shaped cells in the population when SS blood was mixed with HY RBC was 13.5 ± 1.9 % (n=3), which was not significantly different as observed after mixing and exposure for either one minute (12.2 ± 1.3 %) or 25 minutes (12.2 ± 1.4 %) to each other. Similar assessments were made for AA blood mixed with SS blood. In this case the data were obtained after 25 minutes only. When the SS RBC were exposed for 10 minutes to 1% oxygen, 80% of the cells showed a typical sickled morphology.

Table 1. percentage of abnormal cells in the population expected and observed after mixing.

Abnormal morphology (10,000 cells analyzed			
	expected %	observed % 1 minute	observed % 25 minutes
HY + SS	13.5±1.9	12.24±1.3	12.2±1.4
AA + SS	13.7±2.2		12.4±1.4

Conclusions

The data show that the expected percentage of abnormal cells did not significantly ($p>0.05$) differ from the actual observed abnormal cells in the population when SS blood was mixed with either HY or AA RBCs for exposure time of either 1 or 25 minutes. In summary, the data collected did not show an increase in abnormal RBC morphology (sickling) when hypoxic RBCs were mixed in a one to one ratio with sickle blood in an in vitro model that mimicked RBC transfusion.

P-NE-13

Measurement of Platelet Function and Select Cytokines of Whole Blood Stored in Novel Hypoxic Platform

Camille Van Buskirk, MD[1], Nga Thai, MLS[1], Ranee Wannarka Farlinger, MLS[1], Scott A. Hammel, MLS, MA[1], Anderson Sarah, MSM[1], Roy Dyer, PhD[1], Muthuvel Jayachandran, PhD[1], Tatsuro Yoshida[2]

(1)Mayo Clinic, Rochester, MN, USA (2)Hemanext, Lexington, MA, USA

Background

Whole blood (WB) has regained favor in treatment of massively bleeding patients in military and civilian settings. 1 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. 2,3 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 pO2=5-15 mmHg and unit was stored in O2-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 202vs. H 142, $p=0.0028$. PDGF-BB (ng/mL) showed a similar pattern: W1– C 19; H 17, $p<0.19$, and W3–C 3; H 26, $p<0.001$.

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.

P-NE-17

Long-Term Hypoxic Storage of Red Blood Cells Results in Amelioration of Lesion Hallmarks and Increased In Vivo Recovery at 24 Hours Post-Transfusion

Dr. José A. Cancelas, M.D., Ph.D.[1], Pamela H. Whitley[2], Neeta Rugg[1], F Bernadette West[3], Michael S. Wellington[2], Scott Brooks[4], Dr. Matthew S. Karafin[5], Michael Dioguardi[6], Tatsuro Yoshida[6] and Andrew Dunham[6]

(1)Hoxworth Blood Center, Cincinnati, OH, (2)American Red Cross, Norfolk, VA, (3)American Red Cross Blood Services, Farmington, CT, (4)BloodCenter of Wisconsin, Milwaukee, WI, (5)Versiti, Milwaukee, WI, (6)Hemanext, Lexington, MA

Background

Oxidative damage, dysmetabolism and decreased levels of 2, 3-diphosphoglycerate (2,3-DPG) affect red blood cell (RBC) survival and the affinity of Hb for oxygen are relevant hallmarks of RBC storage lesion, especially in specific clinical contexts. We hypothesized that oxygen and carbon dioxide reduction followed by hypoxic storage (Hemanext) would result in amelioration of RBC lesion assessed at the end of their shelf-life and at the shelf-life mode (21 days).

Study Design

A pivotal, prospective, randomized, two-arm, crossover, three-center trial was conducted to evaluate Hemanext hypoxic processing system for meeting standard FDA acceptability criteria for RBC storage. The Test arm consisted of whole blood (WB)-derived, leukoreduced RBC in AS-3 additive processed at room temperature with the Hemanext system for 3 hours to achieve hypoxic/hypocapnic state within 12 hours of phlebotomy which was maintained hypoxic during storage for up to 43 days at 1-6°C (Test). Unprocessed units (Control) were stored within 8 hours under conventional storage conditions. Subjects (N=100) donated CP2D WB (500 ± 50 mL) and a minimum of 93 pairs of RBC units per arm were analyzed for in vitro quality parameters. For in vivo analysis at end of storage, RBCs from 19 test subjects and 21 control subjects (14 paired) from two sites were radiolabeled with 51-Cr/99-Tc(m), autologously transfused, and analyzed for 24-hour recovery. Differences between the Test and Control groups were analyzed using the paired t-test.

Results

Paired analyses of 24-hour in vivo recoveries on day 43 was 89.3±4.5% and 84.8±6.2% for the test and control, respectively). Significantly higher levels of 2,3-DPG and adenosine 5'-triphosphate (ATP) were maintained for the Test by day 21 and 42/43 of storage (Table 1). Percent hemolysis was similar in both groups.

	Storage conditions Mean ± SD		P value
***In vitro* Parameters on day 21**	**Hypoxic**	**Conventional**	
ATP (µmol/g Hb)	4.86±0.06	4.19±0.57	p<0.001
2,3DPG (µmol/g Hb)	8.14±4.14	0.47±0.37	p<0.001
Hemolysis (%)	0.19±0.01	0.18±0.01	N.S.
In vitro Parameters on day 42/43			
ATP (µmol/g Hb)	3.55±0.75	3.33±0.71	p<0.001
2,3DPG (µmol/g Hb)	0.87±0.87	0.43±0.57	p<0.001
Hemolysis (%)	0.29±0.17	0.29±0.15	N.S.
51-Cr/99-Tc(m) 24h Recovery (%)	89.3±4.5	84.8±6.2	p<0.05

N.S.: not significant

Conclusions

These data demonstrate that RBCs preserved in a user-friendly, self-contained hypoxic storage system are superior than the conventionally stored RBCs and may provide more viable RBCs for transfusion at 6 weeks of storage.

Disclaimer: The 51-Cr/99-Tc(m) labeling at Site 3 occurred without prior RDRC approval and was done at a site that did not meet GMP standards.

BBC61

Determination of %SO2 in More Than 1300 Fresh Erythrocyte Concentrates by Resonance Raman Spectroscopy

Herbert G. Korsten[1], Tatsuro Yoshida[2] and Dirk de Korte*[1,3]

(1)Department of Product and Process Development, Sanquin Blood Bank (2)New Health Sciences Inc. (3)Sanquin Research and Landsteiner Laboratory

Background/Case Studies

In support of research on improving erythrocyte storage under low oxygen tension (pO2), hemoglobin oxygen saturation (%SO2) in fresh erythrocyte concentrates (RCCs) was measured non-invasively through the wall of RCC bag using Resonance Raman Spectroscopy (RRS). Surprisingly, a large variation in %SO2 of fresh (0-2 days) RCC was found in a pilot study. For further analysis of this finding, a large number of freshly prepared RCCs at Sanquin Blood Bank were analyzed with the RRS device. To determine relationships between observed variation in %SO2 and donor/donation variables we used external oxygen saturation measurement with RRS of > 1300 fresh RCCs immediately after component preparation.

Study Design/Methods

For 1337 fresh RCCs, the %SO2 was analyzed with RRS device (A3U11 Microvascular Oximeter, Pendar Medical). This device was validated by analyzing the %SO2 of 12 RCCs in comparison with %SO2 measurements on a qualified blood gas analyzer (Radiometer ABL90 FLEX). Additionally, to investigate whether low or high %SO2 values had an effect on the shelf life, RCCs were sampled on day 1 and 35 for blood gas and CBC (Sysmex XT2000) as well as for hemolysis and ATP content.

Results/Findings

The results of the %SO2 measurements with the RRS device corresponded to the %SO2 measurements on the blood gas analyzer with a difference of 0.3% ± 1.5%. The %SO2 data from 1337 donors were compared with donor/donation data such as blood pressure, time of donation, donation duration, as well as individual donor characteristics (weight, height, calculated BMI, age, Hb and gender). The %SO2 data from the 1337 RCCs showed a binomial distribution, with two peaks, strongly influenced by gender of the donors; men (56% of subjects; %SO2 = 65.0 ± 16.0) and women (44% of subjects; %SO2 = 52.7 ± 18.6). The other donor parameters showed no clear effects on %SO2. Cell counts, hemolysis (day 1, 0.09%± 0.02; and day 35, 0.36 %± 0.22) and ATP (day 1, 5.69 µmol/gHb ± 0.43/; and day 35, 3.19 µmol/gHb ± 0.48) showed normal expected values, regardless of whether the %SO2 value was high or low.

Conclusions

The %SO2 measured by the RRS laser device correspond to % SO2 measured on a blood gas analyzer. The use of the RRS device had no adverse effect on the in vitro quality of erythrocytes during storage in a small test group of 12 RCCs and there was no link between in vitro quality and %SO2 value immediately after collection. The mean %SO2 for female donors was lower than for male donors. This cannot be explained on the basis of other available donor/donation data. Further research is needed to explain this difference.

NIT29

Effects of Gamma Irradiation on the Growth of T-Lymphocytes and Quality of Red Blood Cells Stored in Oxygen-Reduced Condition

Loren Fast[1], Samuel Sowemimo-Coker*[2] and Andrew Dunham[2]

(1)Rhode Island Hospital (2)Hemanext

Background/Case Studies

Hemanext has developed a technology for long term storage of red blood cells (RBCs) in AS3 under oxygen-reduced storage condition. Storage of RBCs under this condition has been shown to improve the quality of the cells for transfusion. White blood cells (WBCs) in RBCs for transfusion may be responsible for causing transfusion associated graft versus host disease (TA-GVHD) in immunocompromised patients. Gamma irradiation and leukocyte-reduction of blood products for transfusion are currently being used to prevent this serious adverse effect of blood transfusion. It is unknown if the effectiveness of gamma irradiation for WBC abrogation will be affected by the reduced oxygen content in Hemanext RBC. Therefore, the present study was designed to determine the effects of Hemanext RBC storage condition on the quality of red blood cells and growth and proliferation of T-lymphocytes following gamma irradiation.

Study Design/Method

Two units of fresh leukocyte reduced red cell concentrates (LR-RCC) in AS3 were obtained from Rhode Island Blood Center (RIBC). The two units were pooled together to create 600mL of homogenous pool of LR-RCC. Peripheral blood mononuclear cells (PBMNC) were isolated from one or two units of ABO matched non-leukocyte red cell concentrates (NLR-RCC) using Ficoll gradient and CD3 antibody coated magnetic beads per manufacturer's instructions for use. The PBMNC isolated from the aliquot of blood were added back to the pooled LR-RCC at a final concentration of 2×105/mL. Equal aliquot of 300mL of the pooled unit was added to a conventional PVC red cell storage bag while the other 300mL was de-oxygenatedfor 3 hours at room temperature with Hemanext Red Cell Processing System, transferred into Hemanext bags for storage at 1-6°C for 42 days. In vitro metrics (ATP, 2, 3DPG, hemolysis etc.) of red cell quality were measured before, after processing and during storage. After storage for 7 days, 100 mL were removed from each of the bags and the RBCs remaining in the bags were then exposed to 25 Gy of gamma irradiation per standard protocol at RIBC. PBMNC were isolated from the pre- and post- irradiation samples and tested for their ability to respond to mitogens in a limiting dilution assay.

Results/Findings

The percent oxygen saturation of the hemoglobin (%SO2) in the Hemanext RBC samples were reduced from 57.3 ± 15.2% to 6.9± 2.4% (N=4). The hemolysis in the samples 7 days after gamma irradiation were 0.25 ± 0.18% for control and 0.24 ± 0.09% for Hemanext. The results showed significant growth and proliferation of T-lymphocytes at different levels with non-irradiated control and Hemanext RBCs. The growth and proliferation of Tlymphocytes were inhibited with at least a 4.7 × 104-fold reduction in the frequency of responding T cells following gamma irradiation in the PBMNC in RCC stored in normal conventional or Hemanext RBC storage bags.

Conclusions

These results indicate that the proliferation of WBCs present in RBCs processed with Hemanext red cell processing system and stored in Hemanext storage bags is susceptible to gamma irradiation in a similar fashion to those stored with current conventional storage condition.

ISBT

2020 Abstracts

ISBT Abstract P-229

Hypoxic Storage Improved the Quality and Viscosity of Red Blood Cells in PAGGSM Additive Solution

S Sowemimo-Coker*[1], M Ceniza[1], M Delva-Charles[1], L Omert[1]
(1)Hemanext, Lexington, United States

BACKGROUND

Gamma irradiation of blood products is used to prevent transfusion-associated graft versus-host disease (TA-GVHD) by inhibiting the proliferation of lymphocytes that are implicated in the transfusion reaction. However, gamma irradiation also generates reactive oxygen species which damage the red blood cells (RBCs). Reducing the available oxygen in the RBCs prior to gamma irradiation may reduce the damaging effects of reactive oxygen species on the RBCs. Hypoxic storage of RBCs has been shown to reduce oxidative damage and improve the quality of RBCs.

AIMS

The main objective of the present study was to investigate the effects of hypoxia on the viscoelasticity and other *in vitro* properties of gamma irradiated RBCs in PAGGSM additive solution.

METHODS

Sixteen units of whole blood (500±50mL) were collected into CP2D anticoagulant (Rhode Island Blood Center, RI, USA). Each unit was processed into plasma and a leukocyte-reduced red cell concentrate (LR-RCC) in PAGGSM. For each test, two units (300-340mL each) of ABO matched LR-RCC were pooled together into a 1-liter pooling bag. Equal aliquots from the pool were transferred into bag A for conventional storage and bag B, Oxygen reduction bag (ORB, Hemanext, Lexington, MA, USA). The ORB was agitated at room temperature on a linear agitator at 70rpm for 3 hours to reduce the percent oxygen saturation of the hemoglobin in the RBCs (%SO2) to less than 20%. The oxygenreducedRBCs were transferred into an oxygen impermeable bag (Hemanext) for storage at 1-6°C with unit A for 42 days. The units were gamma irradiated at 25Gy on 14 of storage and then stored until expiry. The following in vitro metrics of RBCs were measured before, after gamma irradiation and during storage: hemolysis, gas panel, extracellular potassium, glucose, lactate, methemoglobin, ATP, 2, 3DPG, osmotic fragility, mechanical fragility and viscoelasticity profile at 40 different shear rates with a Cone plate viscometer (Brookfield-Ametek, Middleboro, MA, USA) in AB+/- plasma at 40% hematocrit. Differences between hypoxic and control RBCs were analyzed with Student “t” test statistic for paired data and $p<0.05$ was considered significant difference between the two treatment conditions.

RESULTS

All the data are the means± standard deviations of 8 independent pools of LR-RCC, n=8. The %SO2 in the hypoxic RBCs on day 1 was 10.6±4 and 17.7% on day 35, compared to 51.6±11.8 on day 1 and 93.0±3.9% on day 35 in the control. The concentrations of 2,3DPG and ATP were significantly higher in hypoxic than in conventionally stored RBCs on Day 35 of storage, $p<0.05$, (2,3DPG, 4.0±1.7 vs 0.8±0.2µmol/g Hb; ATP, 3.9±0.8 vs 3.1±0.7µmol/g Hb) while the viscosities at low and high shear rates were lower in hypoxic RBCs than in conventionally stored cells (at 20 s-1, 11.3 ±1.4 vs 12.1±1.7cP; at 100 s-1, 7.0±0.7 vs 7.5±0.8cP). There were no significant differences in extracellular potassium (57.5±3.2 vs 56.8±3.2 mmol/L) and hemolysis (0.48±0.15 vs 0.45±0.16%) on day 35 of storage $p>0.05$. Hypoxic RBCs also appeared to be more osmotically stable than conventionally stored RBCs up to day 21 of storage (hemolysis at 0.45% NaCl, 48.0±16.3 vs 59.6±18.0%, $p<0.05$).

SUMMARY/CONCLUSIONS

These data suggest that storage of RBCs under hypoxic condition may reduce the deleterious effects of gamma irradiation on RBCs resulting in better quality RBCs for transfusion when compared to conventionally stored cells.

ISBT Abstract P-754

Estimating Oxidative Damage Burden of Red Cell Concentrates in Massive Transfusion

S DePew*[1], N Singh[1], A Dunham[2], R Patel[1], M Marques[1], T Yoshida[2]

(1)Pathology, University of Alabama at Birmingham, Birmingham, (2)Hemanext, Lexington, United States

BACKGROUND

Trauma patients suffering from hemorrhagic shock often require massive transfusion (MT; > 10 units in 24 hr) with packed red blood cells (PRBCs). Although PRBCs can be stored and used for up to 42 days (D), storage lesions accumulate and transfusing older units has been associated with increased patient mortality in multiple observational studies. One major cause of these storage lesions is oxidative reactions causing oxidative damage to the cells, which accumulates with the aging of the unit (Yoshida Blood Transfus. 2019). Recent studies demonstrated that the concentration of oxygen (O2) in PRBC unit varies widely, mostly attributed to donor factors (*ibid* Yoshida 2017). Since O2 is the fuel for the oxidative reactions, O2 content during storage can affect quality of stored PRBC. Due to the need for multiple units, trauma patients requiring MT are at higher risk for increased exposure to transfusion of damaged RBCs stemming from oxidative damage during storage.

AIMS

This study aims to determine the O2 content of PRBC used for MT, as well as to estimate the extent of oxidative stress burden a typical trauma patient might receive.

METHODS

Since more than 98% of O2 is bound by hemoglobin (Hb) in PRBC, Hb O2 saturation levels (SO2) were measured from 135 units of stored PRBCs from MT coolers in the local blood bank. These measurements were obtained non-invasively from the storage bag using a visible reflectance spectroscopy (Moor VMS-OXY, UK). SO2 gradually increases with storage due to O2 permeability of the plastic bag. The SO2 at their collection (D0 SO2) as well as the day of transfusion were calculated (Yoshida 2017) from the age of the units on the day of measurement. To estimate the cumulative O2 exposure, the O2 exposure index, OEI (unit; SO2*D), defined as AUC of SO2 vs. day-in-storage curve, was calculated for each PRBC.

RESULTS

The SO2 of the 135 PRBC at measurement day (9.8±2.7D) varied from 42% - >95%, with a median of 67%. The estimated SO2 at D0 showed wide distribution, varying from 25% - 85%, with a median of 43%. The SO2 increased with age, and the *estimated* SO2 on the day of transfusion (12.8 ±3.7D) ranged 30 - 80%, with the majority of units in 40-65%. Reflecting the wide distribution of D0 SO2, the calculated OEI ranged from 200 - 1,100 SO2*D, with a mean of 574 ± 208. OEI burden in the majority of transfused PRBC was 200 to 1,400 SO2*D.

SUMMARY/CONCLUSIONS

Oxidative damage is fueled by O2 contained within each PRBC. O2 exposure during PRBCs' time on the shelf has cumulative effects, manifesting as storage lesions that may result in observed increases in mortality associated with aged PRBCs. The SO2 distribution of PRBCs packed in the MT coolers exhibited a similar, albeit storage-induced right-shifted distribution compared to the previously observed data for D0-D1 SO2 (51.3±19.8%; n=4912). Consequently, the estimated O2 exposure burden at time of transfusion was more than six-fold, suggesting a wide variability in the quality of PRBCs that trauma patients receive. Prestorage reduction of O2 content with a non-O2permeable PVC storage bag minimizes the O2 burden of stored PRBCs and eliminates one variability in the characteristic of PRBCs for trauma patients. Due to the increased risk of oxidative damage to patients requiring multiple transfusions, it is worth considering that hypoxic storage of PRBCs may reduce morbidity and mortality risk in this population.

ISBT Abstract P-220

Storage of Hypoxic Red Blood Cells in Non-DEHP Storage Bags for 42 Days

S Sowemimo-Coker*[1], M Ceniza[1], M Delva-Charles[1], L Omert[1]
(1)Hemanext, Lexington, United States

BACKGROUND

Hypoxic (HYP) and conventional normoxic (NORM) red blood cells (RBCs) for transfusion are currently stored suspended in additive solution in polyvinyl chloride (PVC) bags plasticized with di(2-ethylhexyl)phthalate (DEHP). Concerns over the potential toxicity of DEHP in humans resulted in a proposal by the European Chemicals Agency to ban DEHP use by the third quarter of 2023. Some of the potential alternatives to DEHP are n-butyl tri-n-hexyl citrate (BTHC), di-isononyl cyclohexane-1,2-dicarboxylic acid (DINCH) and di(2-ethylhexyl)phthalate (DEHT).

AIMS

The main objective of the present study was to determine whether HYP RBCs could be stored in non- DEHP plastic bags and maintain *in vitro* properties of RBC quality that are equivalent to that of conventionally stored NORM RBCs in DEHP bags.

METHODS

Thirty units of whole blood (500±50mL) were collected into CP2D anticoagulant (Rhode Island Blood Center, RI, USA). Each unit was processed into plasma and a leukocyte-reduced red cell concentrate (LR-RCC) in AS3. For each test, six units (300-340mL each) of ABO matched LR-RCC were pooled together into a 1-liter non-DEHP pooling bag. Equal aliquots from the pool were transferred into PVC bags labelled as NORM-DEHP, NORM-DINCH, NORM-BTHC, for conventional storage and non-DEHP bags A, B and C oxygen reduction bags (ORB, Hemanext, Lexington, MA, USA). The ORBs were agitated at room temperature on a linear agitator at 70rpm for 3 hours to reduce the percent oxygen saturation of the hemoglobin in the RBCs (%SO2) to less than 20%. The HYP RBCs were transferred into oxygen impermeable HYP bags labelled as:HYP-DEHP, HYP-DINCH and HYP-BTHC for storage at 1-6°C with units NORMDEHP, NORM-DINCH and NORM-BTHC for 42 days. Several *in vitro* metrics of RBCs quality were measured before and during storage including hemolysis, metabolites, osmotic fragility and viscosity at 40% hematocrit in AB+/- plasma. Differences between HYP and NORM RBCs were analyzed with repeated-measures analysis of variance with Newman-Keuls multiple comparison test.

RESULTS

All the data were the means± standard deviations of 5 independent pools (n=5) of LR-RCC. The %SO2 in the HYP RBCs on Day 0 was 11.4±1.9 and 6.5±2.4% on Day 42, compared to 42.6±5.1 on Day 0 and 89.2±5.1% on Day 42 in NORM RBCs. The concentrations of 2,3DPG and ATP were significantly higher in HYP RBCs than in NORM cells on Day 42 of storage, $p<0.05$, (2,3DPG: NORM-DEHP= 0.50±1.16, HYP-DEHP = 0.86±0.32, HYP-DINCH = 1.02±0.36, HYP-BTHC = 1.60± 0.41 µmol/g Hb; ATP, NORM-DEHP = 3.20±0.35, HYP-DEHP = 3.72±0.19, HYP-DINCH = 3.84±0.31, HYP-BTHC = 3.84±0.26µmol/g Hb) while the methemoglobin levels were significantly lower in HYP cells (NORM-DEHP = 1.06±0.17, HYP-DEHP = 0.62±0.13, HYP-DINCH = 0.64±0.11, HYP-BTHC = 0.60±0.10%), $p<0.05$. There were no significant differences in the viscosities at low and high shear rates $p>0.05$ (at 20 s-1, NORM-DEHP =12.7±3.0, HYP-DEHP = 10.9±1.9, HYP-DINCH = 11.7±3.0, HYP-BTHC = 11.2±2.1cP; at 100 s-1 , NORM-DEHP = 7.1±0.6, HYP-DEHP = 6.9±0.6, HYP-DINCH = 6.9±0.8, HYP-BTHC = 6.9±0.7cP). There were no significant differences in hemolysis (NORM-DEHP = 0.27±0.07, HYP-DEHP = 0.28±0.07, HYP-DINCH =0.30±0.06, HYP-BTHC = 0.31±0.07%), $p>0.05$.

SUMMARY/CONCLUSIONS

These data show that the measured *in vitro* metrics of the quality of HYP RBCs suspended in AS3 and stored in DINCH and BTHC bags for 42 days are either equivalent to or better than that of conventionally stored RBCs in DEHP bags with hemolysis below 0.8% at expiry.

ISBT Abstract P-707

Effect of Varying Post-Transfusion Hemoglobin Increments in Chronically Transfused Patients: A Pharmacokinetic Model

H Hultin* [1], T Yoshida[1], L Omert[1], S Coker[1], M Cannon[1], A Dunham[1]
(1)Hemanext, Lexington, United States

BACKGROUND

Quality of life (QoL) for patients who receive chronic red blood cell (RBC) transfusions is affected by the weakness and fatigue experienced during periods of anemia, the frequency of travel to medical facilities, and the long process they must endure from pre-transfusion testing through transfusion, among other factors. Additionally, surveys reveal that fatigue severity (possibly related to anemia) predicts overall survival differently than prognostic indices. The dose-response to transfusion of an RBC unit is variable and affected by both donor and recipient factors. Evidence indicates that a significant percentage of transfused RBCs clear from circulation within hours after the transfusion and that this effect is worse in patients with inflammation or fever (Wendelbo, Vox Sanguinis 2017).

AIMS

To develop a pharmacokinetic model to illustrate the impact of RBC loss shortly after transfusion on in vivo hemoglobin (Hb) concentrations, as measured by hematocrit, and as a tool to predict optimal intervals for chronically transfused patients.

METHODS

A simple, first-order pharmacokinetic model was employed with the following assumptions: 1. A percentage of effete transfused cells is removed from circulation within a short period of time - percentage defined as xe; 2. Robust transfused cells remain in circulation and are lost at a rate of 0.8% per day (D); 3. Patient continues to lose circulating RBCs at a rate of 0.8% /D; and 4. Patient is not producing their own RBCs. Therefore, where C0 = concentration of Hb infused, Cc = concentration of Hb circulating in the body and λe, λr, λc are the elimination constants for effete, robust and circulating cells respectively, dC, the change in Hb at time t can be expressed as: dC=C0*xe*e^(- λe*t)+C0*(1-xe)*e^(- λr*t)+Cc*e^(-λc*t)-Cc Hb increment data from Wendelbo were modelled to illustrate the difference between Hb loss that is assumed to be a linear process based on two time points versus actual losses over time.

RESULTS

The percentage of transfused RBCs removed rapidly after transfusion significantly impacts transfusion interval and overall Hb Area Under the Curve (AUC). Per the first-order model, as xe increases from 0 – 0.5, transfusion interval decreases from 29.5D to 15.6D, AUC decreases from 39.3 to 15.4 g/dl*D and AUC ratio drops with changes in xe indicating lower patient Hb exposure over time. Utilizing this model, an afebrile patient who requires chronic transfusions whose xe=0.29 will require a new transfusion every 22D while a similar patient with fever and xe=0.52 will require a transfusion every 16D. Applying the model to a patient receiving blood <7D old (xe=0.297) vs one receiving blood 22-42D old (xe=0.704) reveals that decreasing xe 0.407 would save 4 transfusion events and 28 units of blood/yr.

SUMMARY/CONCLUSIONS

The proposed model utilizes a standard, 30D transfusion interval based on 120D RBC lifespan characterized by zero loss of red cells within 24 hours of transfusion. Using reported losses in our model illustrates how the immediate loss of RBCs can result in significant variation in predicted transfusion intervals. Failure to recognize this can impact quality of life and may increase morbidity. This finding illustrates the need for careful monitoring of these patients especially during periods of stress or infection and highlights both the unmet need for standardization of red cell units and clarifying the relationship between RBC quality, Hb increment, and patient outcomes.

Hemanext®

For more information, please visit:

www.hemanext.com

Additional research abstracts presented at other conferences available upon request.

Hemanext is a privately held medical technology company dedicated to improving the quality, safety, efficacy, and cost of transfusion therapy. Our research and development efforts center on hypoxically stored red blood cells (RBCs). The novel Hemanext ONE RBC Processing and Storage System, our initial product offering, is a prescription medical device designed to limit oxygen and carbon dioxide levels in the storage environment.

The information is intended for the US audience. The research involved a product that is for Investigational Use Only. No Hemanext product is cleared or approved by the FDA, nor is any Hemanext product currently available for sale in the United States.