COLLECTION, PRODUCTION AND STORAGE OF BLOOD COMPONENTS

Brief report

Superoxide measurement as a novel probe of red blood cell storage quality

Mitasha S. Palha[1,*], Eric A. Legenzov[1,*], Derek R. Lamb[2], James C. Zimring[3], Paul W. Buehler[2,4], Joseph P. Y. Kao[1]

*Mitasha S. Palha and Eric A. Legenzov have contributed equally to this work and share the first authorship.

[1]*Center for Biomedical Engineering and Technology, and Department of Physiology, University of Maryland School of Medicine, Baltimore, MD, United States of America;*
[2]*University of Maryland, School of Medicine, Center for Blood Oxygen Transport and Hemostasis, Department of Pediatrics, Baltimore, MD, United States of America;*
[3]*University of Virginia School of Medicine, Department of Pathology and Carter Immunology Center, Charlottesville, VA, United States of America;*
[4]*University of Maryland, School of Medicine, Department of Pathology, Baltimore, MD, United States of America*

INTRODUCTION

Standard refrigerator storage causes metabolic, protein, and lipid changes in human red blood cells (RBCs). Depending on processing and donor variability, the resulting changes can negatively impact post-transfusion viability of the RBCs[1]. Donor blood processing is a current topic of research directed toward optimizing methods to assess post-transfusion effectiveness[2,3], while research on donor variability focuses on defining the contributions of donor-specific characteristics, e.g., the exposome, diet, drugs, age, sex, and genetics[4,5]. These factors are now known to be cumulative contributors to changes in the molecular, biochemical and physiological quality of RBCs intended for transfusion. Oxidative stress is a major modifier of normal RBC biology. Within the *ex vivo* setting of refrigerator storage, cumulative oxidative damage may increase because of ineffective or inadequate protective and repair processes[6]. A current hypothesis suggests that, rather than just chronological age, donor RBC differences may accelerate aberrant changes in proteins, lipids, and metabolism that are known to decrease RBC quality[7]. Therefore, new methods for assessing factors that are more determinative of donor blood quality are a logical step toward optimizing blood storage methodology.

One unit of packed RBCs for transfusion contains approximately 60 grams of Hb and 210 mg of iron[8]. Iron must be coordinated within each globin chain's heme to facilitate efficient O_2 transport through allosteric transitioning between oxyHb ($HbFe^{2+}O_2$) and deoxyHb ($HbFe^{2+}$). Within RBCs, $HbFe^{2+}O_2$ is involved in a continuous cycle of spontaneous one-electron oxidations that generates superoxide ($O_2^{\bullet -}$) and metHb ($HbFe^{3+}$) (**Figure 1A**). Autoxidation of erythrocytic Hb proceeds at different rates for the α- and β-chains, and is influenced by Hb concentration, pH, and allosteric effectors[9]. To our knowledge, autoxidation is the only experimentally defined source of $O_2^{\bullet -}$ in stored RBCs. Under conditions of homeostasis, NADH-dependent cytochrome b5 reductase (CB5R, also termed metHb reductase or diaphorase 1) efficiently reduces $HbFe^{3+}$ to $HbFe^{2+}$ to maintain low levels of oxidized Hb, absent congenital CB5R deficiencies[10]. In addition to autoxidation, refrigerator-stored RBCs are primed for oxidative stress due to impaired generation of antioxidant enzyme cofactors NADH and NADPH through glycolysis and the pentose phosphate pathway (PPP), respectively (**Figure 1A**)[11,12].

Murine blood storage offers the advantage of intra-strain genetic homogeneity in proof-of-concept testing. To date, PTR experiments in multiple mouse strains demonstrate metabolic correlates to both fresh and stored RBC[13,14]. Here we hypothesize that X-band electron paramagnetic resonance (EPR) spectroscopy and cyclic hydroxylamine-based

Arrived: 19 October 2022
Revision accepted: 30 November 2022
Correspondence: Paul W. Buehler
e-mail: pbuehler@som.umaryland.edu

Blood Transfus 2023; 21: 422-427 doi: 10.2450/2022.0246-22
© SIMTIPRO Srl

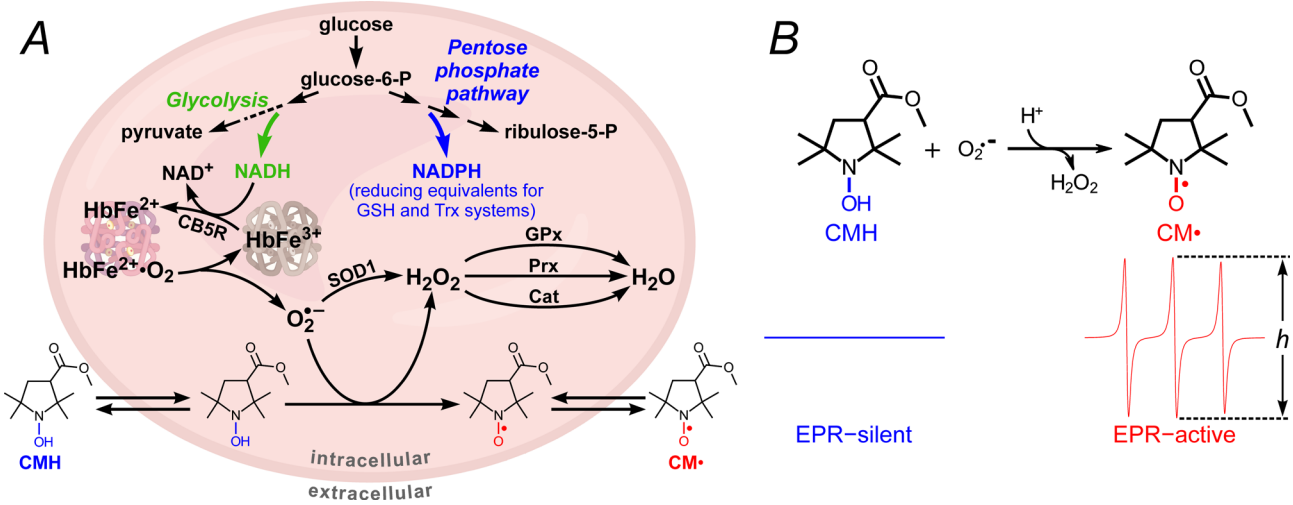


Figure 1 - Key biochemical pathways in erythrocytes and the use of a molecular probe for detecting superoxide by electron paramagnetic resonance (EPR)

A. Glycolysis and the pentose phosphate pathway (PPP) generate the reducing cofactors, NADH and NADPH, respectively. NADPH serves detoxifying systems based on glutathione (GSH) and thioredoxins (Trx). During O_2 transport, a small fraction of oxyhemoglobin ($HbFe^{2+}{\cdot}O_2$) transforms into methemoglobin ($HbFe^{3+}$) with concomitant generation of superoxide ($O_2^{\cdot -}$), which is membrane-impermeant and thus retained in the RBC. Regeneration of ferrous hemoglobin ($HbFe^{2+}$) by CB5R requires NADH. Reactive $O_2^{\cdot -}$ is transformed by superoxide dismutase 1 (SOD1) into hydrogen peroxide (H_2O_2), which is, in turn, detoxified by multiple enzymes, i.e., catalase (Cat), peroxiredoxins (Prx), and glutathione peroxidase (GPx). The cyclic hydroxylamine, CMH**[15]**, is membrane-permeant[16] and thus readily enters RBCs, where it is rapidly oxidized by $O_2^{\cdot -}$ to form the nitroxide, CM•, which is also membrane-permeant[17]. **B**. CMH is EPR-silent, whereas the product of its oxidation, CM•, is EPR active and exhibits a characteristic 3-line EPR spectrum. The height, *h*, of the center line (peak) is used for quantitation in this study.

spin probing of murine RBCs known to store well (C57BL/6J) and poorly (FVB) under refrigeration can identify the generation of $O_2^{\cdot -}$ as a predictive biomarker of oxidative stress. We further suggest that $O_2^{\cdot -}$ kinetics during storage are consistent with reported PTR in these mice[13,14].

MATERIALS AND METHODS

General

Chemicals were purchased from commercial vendors and used without further purification. Xanthine oxidase and superoxide dismutase from bovine erythrocytes were from Sigma-Aldrich (St. Louis, MO, USA). High-resolution mass spectra were acquired on an electrospray ionization spectrometer (AccuTOF-CS, JEOL, Peabody, MA, USA). ^{1}H-NMR spectra were recorded in deuterium oxide on a 400-MHz spectrometer (400 MR, Varian, Palo Alto, CA, USA). EPR spectra were recorded on an X-band spectrometer (EMXnano, Bruker Corp, Billerica, MA, USA). EPR samples were contained in 50-µL borosilicate capillary micropipettes (Drummond Scientific Company, Broomall, PA, USA) whose ends were closed with sealing clay.

Synthesis of 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine hydrochloride (CMH HCl salt): 1-Benzyloxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (0.226 g, 0.776 mmol), prepared from the known compound, 3-methoxycarbonyl-2,2,5,5-tetra-methylpyrrolidinyl-1-oxyl[18], through copper-catalyzed O-benzylation[19], was dissolved in ethanol (10 mL) in a hydrogenation bottle, to which 5% palladium on activated carbon (143 mg) was added. The mixture was agitated overnight under 27 psi H_2 in a hydrogenation apparatus (Parr Instrument Company, Moline, IL, USA). Thereafter, thin-layer chromatography showed complete consumption of starting material. The reaction mixture was filtered through Celite and the filtrate was acidified with concentrated HCl (72 µL). The solvent was removed by rotary evaporation to leave a clear oil which, upon drying under vacuum, crystallized to give a quantitative yield of CMH•HCl. The compound was stored at −20°C under dry argon. ^{1}H-NMR (δ): 1.40 – 1.68 (m, 12H), 2.27 – 2.50 (m, 2H), 3.41 – 3.46 (m, 1H), 3.79 (s, 3H). HRMS: $[M+H]^+$ $C_{10}H_{20}NO_3$ requires 202.1443, observed 202.1436.

Measurement of CMH oxidation by EPR spectroscopy

All samples for EPR measurement contained 1 mM CMH. Other reagents, when used, were at the following concentrations: xanthine oxidase (XO), 0.03 U/mL;

superoxide dismutase (SOD), 5 µM; hypoxanthine (HX), 0.5 mM; hydrogen peroxide (H_2O_2), 1 mM. Cell-free measurements were made in phosphate-buffered saline (PBS) containing 1 mM diethylenetriaminepentaacetic acid (DTPA). For measurements on RBCs, each sample was made by mixing 2 µL of 50 mM CMH•HCl stock solution with 98 µL of RBC suspension (~40% hematocrit) in Citrate Phosphate Dextrose Adenine (CPDA-1, Sigma-Aldrich, St. Louis, MO, USA). Time zero (t=0) is the time at which CMH is mixed into the sample. The amplitude (h) of the center peak of the CM• nitroxide EPR spectrum was converted into concentration (C in µM) by using the calibration equation $C=(h-0.03308)/0.06461$, determined using a series of standard samples.

Murine blood collection and storage

Mouse blood collections were approved by the University of Maryland, Baltimore Institutional Animal Care and Use Committee (IACUC) (protocol # 0520008) and two different strains (C57BL/6J or B6 for short, Jackson Laboratory, Barr Harbor, ME and FVB, Charles River, Worcester, MA, USA) were studied. Blood was collected via cardiac puncture into citrate phosphate dextrose from n=15 mice/strain, pooled, centrifuged, followed by removal of buffy coat and plasma. CPDA-1 (12.5% of the total volume) was added to RBCs (hematocrits 70%). Aliquots were stored in 1.7 mL sterile Eppendorf tubes in a refrigerator at 4-6°C. Measurements were made as four technical replicates from the same pooled RBC tube on day 1 (immediately after processing) and then on days 2, 4, and 8 after processing.

Data analysis and presentation

Least-squares curve fitting and statistical analysis were performed using OriginPro software (OriginLab Corp., Northampton, MA, USA). Results are presented as mean ± SD. Difference between the two strains on the same day was assessed by t-test; within-strain differences over multiple days of storage were assessed through ANOVA with Scheffé *post hoc* test.

RESULTS

CMH oxidation by superoxide

The time course of the reaction of CMH with $O_2^{\bullet -}$ is shown in **Figure 2A** (red symbols and lines, label "XO"). Oxidation of hypoxanthine (HX) by xanthine oxidase (XO) generated $O_2^{\bullet -}$. CMH, which is EPR-silent, was oxidized by $O_2^{\bullet -}$ to CM•, a stable nitroxide with a 3-line EPR spectrum. The spectral peak height was converted to CM• concentration and plotted against time. As oxidation proceeded, CM• accumulated and its concentration rose steadily. Addition of superoxide dismutase (SOD) inactivated $O_2^{\bullet -}$ efficiently, and blocked the conversion of CMH to CM• (**Figure 2A**, green symbols and lines, label "XO+SOD") ($p=3.53\times10^{-5}$).

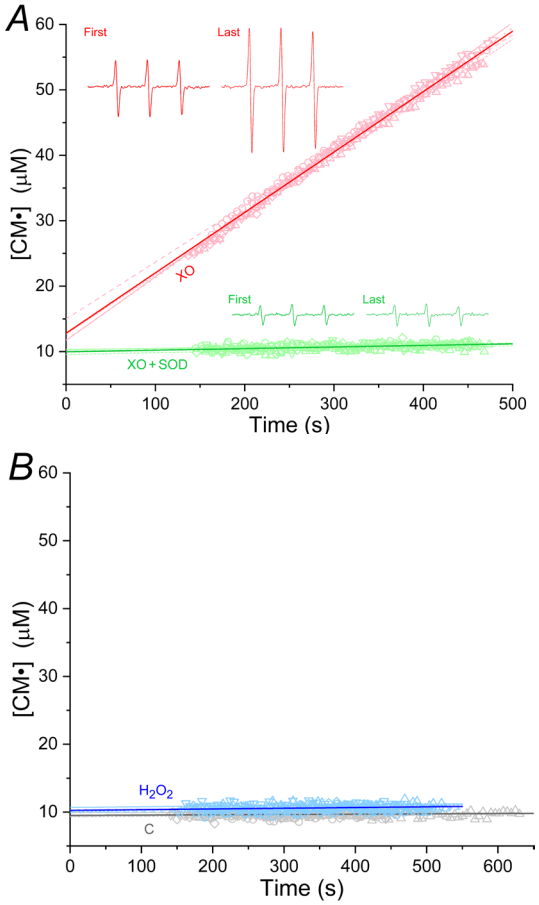


Figure 2 - Reaction of CMH with superoxide and hydrogen peroxide

CMH, which is EPR-silent, reacts with superoxide, generated during the oxidation of hypoxanthine (HX) by xanthine oxidase (XO), to yield CM•, a nitroxide that has a 3-line EPR spectrum. The amplitude of the center spectral peak is converted to CM• concentration and plotted against time. Quadruplicate data sets are plotted with different symbols (○△◇▽) with corresponding least-squares fitted lines (dashed, solid, dotted, short-dashed). The bold solid line is the average of all four least-squares lines in each data set. **A**. Red symbols and lines (labeled "XO"): Time courses of the reaction in replicate samples containing 1 mM CMH, 0.03 U/mL XO, 0.5 mM HX and 1 mM DTPA in PBS. Green symbols and lines (labeled "XO+SOD"): Same samples with 5 µM superoxide dismutase (SOD) added. Insets show the first and last EPR spectra acquired in a typical run; all spectra are on the same scale. **B**. Blue symbols and lines (labeled "H_2O_2"): Reaction time course in samples containing 1 mM CMH, 1 mM H_2O_2, and 1 mM DTPA in PBS. Gray symbols and lines (labeled "C"): Control samples containing only CMH and DTPA in PBS.

Because SOD converts $O_2^{\bullet -}$ to H_2O_2, the XO+SOD result implies that H_2O_2 did not oxidize CMH to CM•. This is confirmed by the experiment shown in **Figure 2B**, where 1 mM H_2O_2 (blue symbols and lines) caused negligible CMH oxidation compared to control (gray symbols and lines, label "C") (p=0.144).

Superoxide generation in red blood cells from FVB and B6 mice during storage

Superoxide measurements using CMH were performed on RBCs of both mouse strains immediately after collection and processing (on Day 1), and on Days 2, 4, and 8 after storage. The results are shown in **Figure 3**. Across all days in storage, CMH oxidation occurred more rapidly in RBCs from FVB mice than in RBCs from B6 mice (compare slopes of linear least-squares fits, **Figure 3A** and **3B**; $p<2.00\times10^{-3}$), implying a higher rate of superoxide production in FVB RBCs. The oxidation rate is a characteristic of each strain and did not vary significantly with days in storage (**Figure 3B**; B6: p>0.414; FVB: p>0.802). A higher rate of superoxide production could increase the steady-state level of superoxide, which would be reflected in higher initial [CM•], i.e., larger values of for the *y*-intercepts of least-squares lines fit to the data. Indeed, the *y*-intercepts are consistently higher for FVB RBCs (**Figure 3C**; p ≤0.0209).

DISCUSSION

Refrigerator storage and human donor characteristics alter metabolic processes that induce RBC injury[6,11]. From a translational research perspective, murine strains are useful models for blood storage and transfusion experiments. Several studies have demonstrated that FVB mice produce RBCs with a poor storage phenotype, based on PTR[13,14,20]. Current regulatory standards require processed human RBCs meet a 24-hour PTR criterion not less than 75% of the infused RBC mass[3]. RBCs of FVB mice

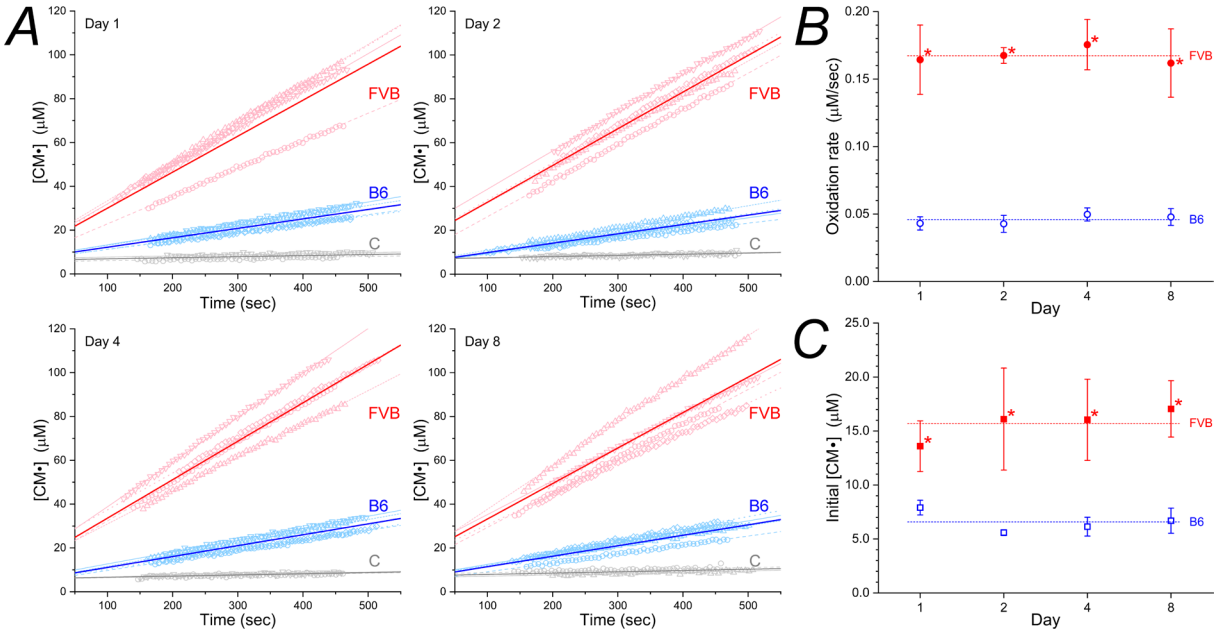


Figure 3 - Superoxide production in FVB and B6 RBCs after different times in storage
A. Comparing oxidation of CMH to CM• in suspension of RBCs after various times in storage; results for FVB are in red and results for B6 are in blue. Quadruplicate data sets are plotted with different symbols (○△◇▽) with corresponding least-squares fitted lines (dashed, solid, dotted, short-dashed). The bold solid line is the average of all four least-squares lines in each data set. Gray symbols and lines (labeled "C") are control measurements in CPDA-1. **B**. The rates of CMH oxidation in FVB and B6 RBCs on various days of storage. The average rate for each strain (the slope of the averaged least-squares lines) is plotted *vs* day in storage. Comparing FVB and B6 shows significant difference on all days ($p\leq2.00\times10^{-3}$, *). Within-strain comparisons across all days show no significant difference (FVB: p>0.802; B6: p>0.414). **C**. Initial concentrations of CM• formed in FVB and B6 RBCs on various days of storage. Average [CM•] for each strain (the *y*-intercept of the averaged least-squares lines) is plotted *vs* day in storage. Comparing FVB and B6 shows significant difference on all days (p<0.0209,*). Within-strain comparisons across all days showed little difference (FVB: p>0.600; B6: p>0.268 except for days 1 *vs* 2, p=0.0139, and 1 *vs* 4, p=0.0661).

have essentially normal lifespans *in vivo*[13, 20,21]; however, storage reduces PTR by as much as 80%[13]. Further, PTR of stored donor FVB mouse RBCs correlates with metabolic profiles that are consistent with cellular oxidative stress, particularly lipid peroxidation[13]. The reactive oxygen species (ROS) responsible for lipid peroxidation are presumed to originate from $O_2^{\bullet -}$; however, their identity remain unknown. Because of the poor-storing RBC phenotype, the present study was designed to evaluate CMH oxidation to CM• in the RBCs from donor FVB and B6 murine strains on days 1, 2, 4 and 8 of refrigerator storage. This proof-of-concept study used X-band EPR measurements to define the potential utility of using CMH oxidation as a probe of $O_2^{\bullet -}$ generation in RBC storage units. We show that CMH does not react with H_2O_2, suggesting specificity for reaction with $O_2^{\bullet -}$. SOD and catalase are the primary sequential systems in RBCs for enzymatic dismutation of $O_2^{\bullet -}$ to H_2O_2, followed by conversion of H_2O_2 to molecular oxygen and water. Therefore, CMH is oxidized during a physiological excess of $O_2^{\bullet -}$. In this study, FVB mice produced greater amounts of $O_2^{\bullet -}$ at the pre-storage measurement and these levels were maintained throughout the duration of storage. Here, stored FVB mouse RBCs showed significantly enhanced rates of CMH to CM• oxidation starting from day 1 of *ex vivo* storage compared to RBCs from B6 mice. Interestingly, in this study a 3-fold greater mean rate of CMH to CM• oxidation was consistently observed in FVB versus B6 RBCs on days 1, 2, 4 and 8 of storage. Further, on each day of measurement, the mean [CM•] was consistently ~9 μM higher in RBCs from FVB mice at the time of CMH addition.

A potential limitation of this study is that a leukoreduction step was not used in our blood processing method. Therefore, residual white cells or platelets could have produced their own $O_2^{\bullet -}$ and thus contributed toward CMH oxidation independent of RBCs. Nonetheless, RBCs from both murine strains were processed the same way; therefore, extraneous cellular $O_2^{\bullet -}$ production would likely be similar. Further, we do not know if the source of $O_2^{\bullet -}$ is its excess production or impaired dismutation in FVB mouse RBCs maintained under refrigerator storage, or if $O_2^{\bullet -}$ is the primary contributor to the poor storage phenotype. Additional preclinical and clinical experiments are critical to understanding the importance of rapid and accurate measurements of ROS in the assessment of donor RBC quality.

CONCLUSIONS

We demonstrate that a cell-permeant molecular probe (CMH) that is oxidized by $O_2^{\bullet -}$ to generate a stable nitroxide (CM•), in conjunction with a compact X-band EPR spectrometer, can quantify superoxide generation in RBCs and differentiate murine strains whose RBCs store well or poorly in the refrigerator. Currently, blood storage is guided only by process control (e.g., collection particulars, temperature of storage, outdate, etc.); transfused RBCs are not subjected to any quality control metrics relevant to PTRs. As such, the described method has the potential to identify RBCs that are likely to store poorly through measurements at time of collection and prior to storage. Applying this method to testing collected and/or stored RBCs has the practical potential to improve clinical outcomes by avoiding transfusion of units that do not meet FDA criteria. This method also has the potential of revealing novel determinants of RBC quality that can inform improvements in blood storage technology.

FUNDING AND RESOURCES

This study was in part funded by The National Institutes of Health, National Heart, Lung, and Blood Institute grants R01 HL161004, R01 HL162120 and R01 HL1159862 to PWB and R01 HL14815, R01 HL146442, R01 HL149714 to JCZ.

AUTHORSHIP CONTRIBUTIONS

PWB, JPYK, and JCZ conceived the project. EAL, MSP, DRL, PWB, and JPYK designed the measurement methodology. MSP and JPYK performed chemical synthesis. MSP, EAL, and DRL conducted the experiments; MSP and EAL analyzed the data. All Authors contributed to the drafting of the manuscript.

DISCLOSURE OF CONFLICTS OF INTEREST

JCZ declares to be consultant for Rubius Therapeutics and Founder and CSO of Svalinn Therapeutics. The other Authors declare no conflicts of interest.

REFERENCES

1. Yoshida T, Prudent M, D'Alessandro A. Red blood cell storage lesion: causes and potential clinical consequences. Blood Transfus 2019; 17: 27-52. doi: 10.2450/2019.0217-18.

2. Ochocinska MJ, Spitalnik SL, Abuhamad A, Bennett-Guerrero E, Carlo WA, Cherukuri M, et al. NIH Workshop 2018: towards minimally invasive or noninvasive approaches to assess tissue oxygenation Pre- and post-transfusion. Transfus Med Rev 2021; 35: 46-55. doi: 10.1111/trf.14435.
3. Vostal JG, Buehler PW, Gelderman MP, Alayash AI, Doctor A, Zimring JC, et al. Proceedings of the Food and Drug Administration's public workshop on new red blood cell product regulatory science 2016. Transfusion 2018; 58: 255-266.doi: 10.1111/trf.14435.
4. D'Alessandro A, Fu X, Kanias T, Reisz JA, Culp-Hill R, Guo Y, et al. Donor sex, age and ethnicity impact stored red blood cell antioxidant metabolism through mechanisms in part explained by glucose 6-phosphate dehydrogenase levels and activity. Haematologica 2021; 106: 1290-302. doi: 10.3324/haematol.2020.246603.
5. Nemkov T, Stefanoni D, Bordbar A, Issaian A, Palsson BO, Dumont LJ, et al. Blood donor exposome and impact of common drugs on red blood cell metabolism. JCI Insight 2021; 6: e146175. doi: 10.1172/jci.insight.146175.
6. Reisz JA, Nemkov T, Dzieciatkowska M, Culp-Hill R, Stefanoni D, Hill RC, et al. Methylation of protein aspartates and deamidated asparagines as a function of blood bank storage and oxidative stress in human red blood cells. Transfusion 2018; 58: 2978-2991. doi: 10.1111/trf.14936.
7. D'Alessandro A, Zimring JC, Busch M. Chronological storage age and metabolic age of stored red blood cells: are they the same? Transfusion 2019; 59: 1620-1623. doi: 10.1111/trf.15248.
8. Agnihotri N, Pal L, Thakur M, Kumar P. The need to label red blood cell units with their haemoglobin content: a single centre study on haemoglobin variations due to donor-related factors. Blood Transfus 2014; 12: 520-526. doi: 10.2450/2014.0231-13.
9. Tsuruga M, Shikama K. Biphasic nature in the autoxidation reaction of human oxyhemoglobin. Biochim Biophys Acta 1997; 1337: 96-104. doi: 10.1016/s0167-4838(96)00156-2.
10. Gibson QH. The reduction of methaemoglobin in red blood cells and studies on the cause of idiopathic methaemoglobinaemia. Biochem J 1948; 42: 13-23. doi: 10.1042/bj0420013.
11. Francis RO, D'Alessandro A, Eisenberger A, Soffing M, Yeh R, Coronel E, et al. Donor glucose-6- phosphate dehydrogenase deficiency decreases blood quality for transfusion. J Clin Invest 2020; 130: 2270-2285. doi: 10.1172/JCI133530.
12. Rogers SC, Ge X, Brummet M, Lin X, Timm DD, d'Avignon A, et al. Quantifying dynamic range in red blood cell energetics: Evidence of progressive energy failure during storage. Transfusion 2021; 61: 1586-1599. doi: 10.1111/trf.16395.
13. de Wolski K, Fu X, Dumont LJ, Roback JD, Waterman H, Odem-Davis K, et al. Metabolic pathways that correlate with post-transfusion circulation of stored murine red blood cells. Haematologica 2016; 101: 578-586. doi: 10.3324/haematol.2015.139139.
14. Waterman HR, Kapp LM, Howie HL, Hod EA, Spitalnik SL, Zimring JC. Analysis of 24-h recovery of transfused stored RBCs in recipient mice of distinct genetic backgrounds. Vox Sang 2015; 109: 148-154. doi: 10.1111/vox.12270.
15. Dikalov SI, Polienko YF, Kirilyuk I. Electron Paramagnetic Resonance Measurements of Reactive Oxygen Species by Cyclic Hydroxylamine Spin Probes. Antioxid Redox Signal 2018; 28: 1433-43. doi: 10.1089/ars.2017.7396.
16. Dikalov SI, Kirilyuk IA, Voinov M, Grigor'ev IA. EPR detection of cellular and mitochondrial superoxide using cyclic hydroxylamines. Free Radic Res 2011; 45: 417-30. doi: 10.3109/10715762.2010.540242.
17. Shen J, Liu S, Miyake M, Liu W, Pritchard A, Kao JP, et al. Use of 3-acetoxymethoxycarbonyl-2,2,5,5-tetramethyl-1-pyrrolidinyloxyl as an EPR oximetry probe: potential for in vivo measurement of tissue oxygenation in mouse brain. Magn Reson Med 2006; 55: 1433-40. doi: 10.1002/mrm.20894.
18. Rozantzev EG, Krinitzkaya LA. Free iminoxyl radicals in the hydrogenated pyrrole series. Tetrahedron 1965; 21: 491-499. doi: 10.1016/S0040-4020(01)82219-5.
19. Matyjaszewski K, Woodworth BE, Zhang X, Gaynor SG, Metzner Z. Simple and efficient synthesis of various alkoxyamines for stable free radical polymerization. Macromolecules 1998; 31: 5955-7. doi: 10.1021/ma9807264 .
20. Kim CY, Johnson H, Peltier S, Spitalnik SL, Hod EA, Francis RO, et al. Deuterated Linoleic Acid Attenuates the RBC Storage Lesion in a Mouse Model of Poor RBC Storage. Front Physiol 2022; 13: 868578. doi: 10.3389/fphys.2022.868578.
21. Howie HL, Hay AM, de Wolski K, Waterman H, Lebedev J, Fu X, et al. Differences in Steap3 expression are a mechanism of genetic variation of RBC storage and oxidative damage in mice. Blood Adv 2019; 3: 2272-85. doi: 10.1182/bloodadvances.2019000605.