Title: Storage following gamma-irradiation affects *in-vivo* oxygen delivery capacity of transfused red blood cells in preterm infants

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ABSTRACT

Background: Gamma-irradiation of red blood cells (RBC) is well-recognized to exacerbate storage lesion formation but the effect of storage following irradiation on *in-vivo* oxygen delivery capacity of transfused RBCs is currently not known.

Study Design and Methods: In 24 anemic preterm infants receiving non-urgent transfusion of irradiated RBCs, we examined cerebral regional tissue oxygenation ($crSO_2$) and time spent with peripheral arterial saturation (SpO_2) less than 88%. Physiological data were obtained immediately before, immediately after and 5 days after transfusion.

Results: We observed linear negative moderate correlations between time since irradiation and the magnitude of change in $crSO_2$ (*r*=-.60, 95%CI: -.81 to -.27, *P*=.0018) and time spent with SpO_2<88% (*r*=-.42, 95%CI: -.71 to .003, *P*=.04) immediately after transfusion. In infants (n=9) who received fresher RBCs (irradiated <10 days before transfusion), there was a sustained increase in mean $crSO_2$ up to 5 days after transfusion (3.0%, 95%CI: 0.3-5.7%, *P*=.04). Conversely, in infants (n=15) who received older RBCs (irradiated ≥10 days before transfusion) there were negligible changes in $crSO_2$ following transfusion at any time point.

Conclusion: Our findings indicate that storage following gamma-irradiation may have a detrimental effect on the oxygen delivery capacity of RBCs given to anemic preterm infants.

KEY WORDS

Transfusion Practices (Neonatal, Pediatrics), Blood Component Preparations, RBC Transfusion

INTRODUCTION

Preterm infants with extremely low birth weight almost invariably require multiple transfusions of red blood cells (RBC), and they represent a group with one of the highest transfusion requirement within the hospital setting.[1] Allogenic RBCs are widely used to treat anemia of prematurity in order to restore cardiorespiratory stability, and to optimize growth and later neurodevelopment. However, there is evidence that implicates transfusion of RBCs in the pathophysiology underpinning preterm-associated comorbidities.[2,3] Additionally, preterm-associated brain injury may in part reflect inadequate oxygen delivery to the brain during a crucial phase of development.[4]

Preterm infants are intrinsically immunocompromised. Therefore, gamma-irradiation of leukoreduced RBCs is used to prevent transfusion-associated graft-versus-host disease (TA-GvHD).[5,6] However, gamma-irradiation also exacerbates 'storage lesion' formation and accelerates biochemical, structural and rheological changes in stored RBCs.[7-11]

According to the current international guidelines on gamma-irradiation, it is safe to store irradiated RBCs for up to 14 days (up to 28 days in the USA).[12-14] While these guidelines are based primarily on the acceptable levels of hemolysis and extracellular potassium concentrations there is a paucity of literature on the *in-vivo* efficacy of irradiated and stored RBCs. Therefore, the present study aims to examine the effects of storage following irradiation on the *in-vivo* oxygen delivery capacity of transfused RBC. We hypothesized that there is a correlation between time since irradiation and the magnitude of change in cerebral regional oxygenation, and in the frequency and duration of peripheral arterial desaturation, and that these effects are not explained by time since donation of RBCs.

MATERIALS AND METHODS

Study population

Twenty-four preterm medically stable infants receiving transfusion of RBCs in Wellington Neonatal Intensive Care Unit, NZ were recruited prospectively into this observational study between May and November 2016. All participants received 15ml/kg of leukoreduced, gamma-irradiated (25Gy) RBCs from cytomegalovirus negative donors, stored at 2-5^{o}C in the saline-adenine-glucose-mannitol (SAGM) solution. Infants on invasive respiratory support, undergoing treatment for systemic infections, or those who had hemodynamically significant patent ductus arteriosus or edema (due to potential interference with signal acquisition) were excluded from the study. Only one transfusion episode per participant was studied. No participant received additional transfusion during the study period.

Study protocol

Cerebral regional tissue oxygenation ($crSO_2$)

Spatially-resolved Near Infrared Spectroscopy (Sensmart Model X-100, Nonin, USA) was applied to measure cerebral regional tissue oxygenation ($crSO_2$) at a sampling rate of 0.25Hz for 3hr at the following time points in relation to transfusion: immediately before, immediately after and 5 days after. In all cases, a neonatal sensor with light penetration depth of 25mm (EQUANOX 8004CB-NA Advanced, Nonin, USA) was placed on the left forehead avoiding hair and the midline.

Peripheral arterial saturation (SpO_2)

Overnight pulse oximetry study (Masimo SET Radical-7/8, Masimo Corporation, USA) was performed for 12hrs at the following time points in relation to transfusion: immediately before, immediately after and 5 days after. A neonatal sensor (LNCSNeoPt/NeoPt-3, Masimo

Corporation, USA) was placed on a hand or a foot, and was rotated between limbs every 4hrs as per local institutional protocol. Peripheral arterial saturation (SpO_2) signals were averaged over 2-4 seconds, and the percentage time spent with SpO2 less than 88% for each study epoch was downloaded using the PROFOX software (PROFOX Associates Inc., USA).

Statistical analysis

Prism 7 (GraphPad Software, Inc., USA) and SPSS v24 (IBM, USA) were used for statistical analysis. Pearson's r correlation coefficients were used to quantify the degree of linear association between two variables. Multivariable models were also used to derive partial correlation coefficients (adjusted for selected potential confounders). Linear mixed models with repeated measures were used to assess the main, and interaction, effects of time since irradiation of RBC ('RBC effect') and time since transfusion ('time effect') on $crSO_2$ and SpO_2. Normal distribution of model residuals was confirmed using a histogram and the D'Agostino & Pearson normality test. $P<.05$ was considered statistically significant.

Ethics

Prospective ethical approval was granted by the Health and Disability Ethics Committees, New Zealand (ref: 16/CEN/18).

RESULTS

Mean (range) gestational age of the participants was 26^{+6} weeks (23^{+3}–32^{+2} weeks) and corrected postnatal age, 32^{+4} weeks (26^{+1}–40^{+5} weeks). Mean birth weight was 953g (595–2050g), and the weight prior to transfusion was 1572g (820–2850g). Mean hemoglobin count and hematocrit ratio prior to transfusion were 87g/L (71–108g/L) and 0.26 (0.22–0.33)

respectively. Mean age of transfused RBC was 10 days (6–13 days) since gamma-irradiation, and 17 days (10–24 days) since donation.

Linear negative correlations were observed between *time since irradiation* and changes in $crSO_2$ (r=-.60, 95%CI: -.81 to -.27, P=.0018), and percentage reduction in time spent with SpO_2<88% (r=-.42, 95%CI: -.71 to -.003, P=.04), from immediately before to immediately after transfusion (Fig. 1a & 1b). The correlation between *time since donation* and change in $crSO_2$ was not statistically significant (r=-.31, 95%CI: -.61 to .10, P=.14) (Fig. 1c). Multivariable linear regression analysis showed a significant relationship between the change in $crSO_2$ with *time since irradiation* (slope -.88%/day, P=.007) but not with *time since donation* (slope .016%/day, P=.92).

Furthermore, linear mixed model analysis demonstrated that infants who received RBCs stored for <10 days since irradiation ('fresher irradiated RBCs') had a greater increase in $crSO_2$ following transfusion vs those who received RBCs stored for ≥10 days since irradiation ('older irradiated RBCs') (Fig. 2a). In the fresher irradiated RBC group, an increase in $crSO_2$ was observed immediately after transfusion (3.7%, 95%CI: 2.0–5.4%), and this increase was sustained up to 5 days after transfusion (3.0%, 95%CI: 0.3–5.7%) (Fig. 2a). Similar trends were seen with time spent with SpO_2<88%, with a significant reduction at 5 days in the fresher irradiated RBC group (Fig. 2b). In the older irradiated RBC group, no significant change in $crSO_2$ or time spent with SpO_2<88% was observed at any time points.

In this sub-analysis, infants who received the fresher irradiated RBCs had a greater corrected postnatal age (Table 1). Consequently, they were heavier and fewer infants in this group required positive pressure respiratory support at the time of transfusion (Table 1). As

corrected postnatal age, weight at the time of transfusion and requirement for respiratory support are dependent variables, in the multivariable analysis we considered requirement for respiratory support as the most significant confounder. When ~~the difference in requirement for respiratory support~~this was accounted for, the partial correlation between *time since irradiation* and changes in $crSO_2$ remained significant (r=-.50, 95%CI: -.76 to -.10, P=.014). Other clinical characteristics and hematological values prior to transfusion did not differ between the groups (Table 1).

DISCUSSION

Our findings suggest that the efficacy of transfused RBCs, defined in the current study as their ability to improve cerebral tissue oxygenation and to reduce time spent with SpO_2<88% in anemic preterm infants, diminishes over time following gamma-irradiation. Furthermore, transfusion of RBCs stored for 10 days or longer following gamma-irradiation led to negligible change in cerebral tissue oxygenation immediately or 5 days after transfusion.

In-vivo and *in-vitro* studies have previously demonstrated that storage of RBC results in alterations to the integrity and function of transfused RBCs.[15-20] However, a number of clinical trials and meta-analyses concluded that transfusion of 'fresher' RBCs did not lead to any difference in key clinical outcomes.[21-23] To date, these studies have focused on the *time since donation to transfusion* and have used less direct measures of RBC function.

Gamma-irradiation of RBCs is practiced widely across the world to protect the transfused immunocompromised patients from the potentially life-threatening complication of TA-GvHD[5,6]. Recent *in-vitro* studies have shown that the storage lesion is exacerbated by gamma-irradiation and that it worsens during subsequent storage.[7-11] In particular, reduction

in intracellular 2,3 di-phosphoglycerate and adenosine tri-phosphate levels, and the loss of erythrocyte deformability and elasticity are concerning for oxygen transport physiology. Limited *in-vivo* data have demonstrated partial recovery of the biochemical compositions of irradiated and stored RBCs following transfusion.[24,25] However, evidence on *in-vivo* efficacy of irradiated and stored RBCs remains unknown.

The results of the current study indicate that storage following gamma-irradiation, even within the timeframe recommended by international guidelines, may have a detrimental effect on the *in-vivo* oxygen delivery capacity of transfused RBCs at least in preterm infants. The study was limited by a small sample size and heterogeneous cohort. Further research is required to investigate this effect in a prospective randomized controlled trial, and to determine the safety and efficacy of current international transfusion guidelines with regards to irradiation and storage of RBCs.

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Authorship contributions

Dr. Saito-Benz conceptualized and designed the study, obtained ethics approval, collected and analysed data, and drafted the initial manuscript.

Dr. Murphy assisted in data analysis and manuscript revision.

A/Prof. Tzeng assisted in designing the study, data analysis and manuscript revision.

Prof. Atkinson assisted in data analysis and manuscript revision.

Dr. Berry had an overall supervisory role in the study, and assisted in conceptualizing and designing the study, data analysis and manuscript revision.

All authors have approved the final manuscript as submitted.

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Figure legends

Figure 1: Relationships between storage duration and cerebral regional oxygenation ($crSO_2$) and reduction in time spent with SpO_2<88%. Pearson's Product-moment correlation coefficients (r) are reported along with the respective 95% confidence intervals. (a) Negative correlation was observed between *time since irradiation* and change in $crSO_2$ from immediately before to immediately after transfusion. (b) Similarly, negative correlation was observed between *time since irradiation* and change in percentage reduction in time spent with SpO2<88%. (c) No correlation was observed between *time since donation* and change in crSO2.

Figure 2: Comparison of fresher irradiated RBCs and older irradiated RBCs ('RBC effect') on cerebral regional oxygenation ($crSO_2$) and time spent with SpO2<88%. Nine infants received RBCs stored for less than 10 days since irradiation (fresher irradiated RBCs), and 15 infants received RBCs stored for 10 days or more since irradiation (older irradiated RBCs). (a) There was a significant interaction between RBC and time effects for $crSO_2$. A sustained increase in $crSO_2$ up to 5 days after transfusion was observed in infants who received fresher irradiated RBCs. No significant change in $crSO_2$ was observed at any of the time points in infants who received older irradiated RBCs. (b) A significant reduction in the time spent with SpO_2<88% was seen 5 days after transfusion in infants who received fresher irradiated RBCs. However, no significant interaction between RBC and time effects was observed. Data is presented in fig. 2a&b as means ± 95% confidence interval. * denotes p<0.05.

Table 1: Comparison of participants' characteristics prior to transfusion

	Time since irradiation of red blood cells		
Participants' characteristics prior to transfusion	Fresher irradiated (n=9)	Older irradiated (n=15)	*p* value
Gestational age (weeks)	27 (23–32)	26 (23–30)	0.31
Corrected postnatal age (weeks)	35 (29–40)	31 (26–36)	0.01*
Birth weight (g)	1122 (595–2050)	865 (615–1310)	0.13
Current weight (g)	2071 (890–2850)	1439 (820–2456)	0.02*
Respiratory support			
CPAP/SiPAP	4 (44%)	14 (93%)	<0.01*
High Flow	1 (11%)	1 (7%)	0.70
FiO_2 > 30%	2 (22%)	7 (47%)	0.23
Furosemide (1mg/kg) use	0	3 (20%)	0.15
Haematology			
Haemoglobin (g/L)	85 (71–103)	88 (78–108)	0.35
Haematocrit (ratio)	0.25 (0.22–0.29)	0.27 (0.24–0.33)	0.10

Data are presented as mean (range) for continuous variables, and n (%) for categorical variables. CPAP: continuous positive airway pressure therapy, SiPAP: synchronized inspiratory positive airway pressure therapy, FiO_2: fractional inspired oxygen. * denotes statistical significance at $p<0.05$