Autologous Infant and Allogeneic Adult Red Cells Demonstrate Similar Concurrent Post-Transfusion Survival in Very Low Birth Weight Neonates

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Objective Based on the hypothesis that neonatal autologous red blood cell (RBC) survival (RCS) is substantially shorter than adult RBC, we concurrently tracked the survival of transfused biotin-labeled autologous neonatal and allogeneic adult RBC into ventilated, very low birth weight infants.

Study design RBC aliquots from the first clinically ordered, allogeneic adult RBC transfusion and from autologous infant blood were labeled at separate biotin densities (biotin-labeled RBC [BioRBC]) and transfused. Survival of these BioRBCs populations were concurrently followed over weeks by flow cytometric enumeration using leftover blood. Relative tracking of infant autologous and adult allogeneic BioRBC was analyzed by linear mixed modeling of batched weekly data. When possible, Kidd antigen (Jka and Jkb) mismatches between infant and donor RBCs were also used to track these 2 populations.

Results Contrary to our hypothesis, concurrent tracking curves of RCS of neonatal and adult BioRBC in 15 study infants did not differ until week 7, after which neonatal RCS became shortened to 59%-79% of adult enumeration values for uncertain reasons. Analysis of mismatched Kidd antigen RBC showed similar results, thus, confirming that BioRBC tracking is not perturbed by biotin RBC labeling.

Conclusions This study illustrates the utility of multidensity BioRBC labeling for concurrent measurement of RCS of multiple RBC populations in vivo. The similar RCS results observed for neonatal and adult BioRBCs transfused into very low birth weight infants provides strong evidence that the circulatory environment of the newborn infant, not intrinsic infant-adult RBC differences, is the primary determinant of erythrocyte survival. *(J Pediatr 2015;167:1001-6)*.

Trial registration Clinicaltrials.gov: NCT00731588.

Severe anemia is a common clinical problem among critically ill infants, particularly those born prematurely.[1] As a result, these patients are among the most commonly transfused of all patient groups.[2] Cited among contributors to the anemia that characteristically develops during early infancy in both preterm and term infants[1,3-6] is the shorter red blood cell (RBC) lifespan observed in both premature (30-50 days) and full term infants (60-70 days) compared with normal adults (110-130 days).

RBC survival (RCS) has been most commonly and accurately performed by direct measurement of labeled populations of RBC in the circulation.[7] In the past, labeling has been done using one of several radioactive isotopes (eg, ^{51}Cr, ^{32}P, and ^{99m}Tc[7]). The major safety problem with this is radiation exposure. Since the 1970s, the use of radioactive compounds in research studies involving vulnerable patient groups such as fetuses, infants, children, and pregnant women has been deemed unacceptable. To overcome this problem and the problem of RBC surface elution of some radioisotopes,[8] labeling RBC with biotin (biotin-labeled RBC [BioRBC]) has been advocated as a practical, reliable, and accurate method for measuring RCS in vulnerable patient groups.[9,10] Furthermore, biotin labeling has the capability of concurrent measurement of RCS of more than 1 RBC population.

BioRBC	Biotin-labeled RBC
Hb	Hemoglobin
RBC	Red blood cell
RCS	Red blood cell survival
VLBW	Very low birth weight

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Supported by US Public Health Service National Institutes of Health (P01 HL046925), the Thrasher Research Fund (0285-3), and the National Center for Research Resources (UL1RR024979, UL1TR000039, and 1S10 RR027219). The Sysmex XE-2100 automatic hematology analyzer used in this study was provided on an on-loan basis from Sysmex Corporation (Kobe, Japan). J.W. serves as a paid consultant for HemoGenix and holds a loan agreement with Sysmex Corporation (Kobe, Japan) for the use of the XE-2100 hematology analyzer in his research lab. D.M. serves as a consultant for MedDay Pharmaceutical. The other authors declare no conflicts of interest.

Portions of this study were presented at the American Association of Blood Banks meeting, Denver, CO, October 12-15, 2013.

http://dx.doi.org/10.1016/j.jpeds.2015.08.028

In the present study, we employed multidensity biotin RBC labeling to concurrently track survival of neonatal autologous and adult allogeneic RBC in very low birth weight (VLBW) infants. Based on the preponderance of RCS studies in infants prior to ^{51}Cr use being curtailed in 1970[11] and RCS in normal adults,[9] we hypothesized that allogeneic BioRBC from adults would survive about twice as long as autologous newborn BioRBC in VLBW infants.[11] We further hypothesized that measurements based on Kidd antigen mismatches would demonstrate the same difference in VLBW infants.

Methods

Prior to initiation, this study received approval from the appropriate Institutional Review Boards, with informed written parental consent. Approval included permission to administer Kidd antigen-mismatched RBC.

We enrolled a prospective, convenience sample of newborn infants receiving their first RBC transfusion based on clinical indications. Included were VLBW infants <31-week gestation requiring mechanical ventilation with survival anticipated. Infants with major congenital anomalies were excluded.

Fresh (<7 days old), AS-3, leukoreduced (Leukotrap RC System; Medsep, Covina, California), irradiated (3000 cGy) blood bank RBC units were tested for Kidd antigen mismatch between the adult donor and the infant recipient. Once the RBC transfusion was ordered, the designated unit had routine blood bank volume reduction by centrifugation to achieve a packed RBC volume of ~0.80. Standard practice at our institution is to transfuse infants with 15 mL/kg of packed RBC. For this study, the first 14 mL/kg of allogeneic donor RBC was transfused as usual by infusion over 3-4 hours. The remaining 1 mL/kg of the clinically ordered allogeneic packed RBC transfusion (plus an additional 2.8 mL of allogeneic packed RBC required following biotinylation for culture and hematologic analysis) and 0.5-3 mL/kg of whole blood from the study infant was biotinylated at 2 discrete low biotin densities between 6 and 36 μg/mL.

Following RBC biotinylation, each BioRBC population was washed, and the supernatant fractions discarded. The washed neonatal autologous and adult donor allogeneic packed BioRBC populations (hematocrits ~45% and ~80%, respectively) were each passed through an 18-micron filter (Hemo-Nate; Utah Medical Products, Midvale, Utah) to remove microaggregates. Immediately after the 14 mL/kg clinical transfusion, the final 1 mL/kg of adult donor BioRBC and the ~1 mL/kg of neonatal autologous BioRBC were infused separately in less than 10 minutes.

Beginning at 24-hour post-transfusion, leftover anticoagulated whole blood from clinically ordered laboratory testing was salvaged up to 4 times per week. Samples were analyzed for the proportions (ie, enrichment) of the 2 BioRBC populations relative to unlabeled RBC and for the proportion of RBC based on Kidd antigen mismatches as described below.

Biotinylation of RBC

RBC were biotinylated as described previously.[10] Briefly, RBC were washed with a carbonate-buffered dextrose sodium phosphate wash solution to remove plasma proteins and resuspended at a hematocrit of 25%. Hematocrit adjustment was verified using the Sysmex XE-2100 (Sysmex, Kobe, Japan). Subsequently, RBC were incubated with the biotinylation reagent, Biotin 3-sulfo-N-hydroxysuccinimide ester (EZ-LINK Sulfo-NHS-Biotin; Pierce Chemical, Rockford, Illinois), at 2 separate concentrations between 6 and 36 μg of sNHS-biotin per mL packed RBC.

Quantitative Flow Cytometric RBC Analysis

BioRBC. The enrichment proportions of the 2 populations of BioRBC relative to the total number of RBC counted was determined in triplicate on 10 μL of salvaged whole blood. These measurements were made by flow cytometric enumeration of 1×10^6 total cells using a FACSCalibur or Fortessa flow cytometer (BD Biosciences, San Jose, California).[9] For samples with slightly overlapping fluorescent intensity histograms, mixture modeling was used to calculate the proportion of cells from each RBC population. The lower limit of quantitation for individual BioRBC density peaks is 0.06% of total RBC.[12]

Kidd RBC Antigen Mismatch. For analysis of Jka or Jkb RBC by flow cytometry, triplicate 3 μL aliquots of the blood samples were washed and incubated with either anti-Jka or anti-Jkb primary antibody (Immucor Inc, Norcross, Georgia) based on previous antigen typing. This primary antibody incubation was followed by incubation with a secondary goat antibody to human IgG conjugated with the fluorescent dye Alexa Fluor 488 (H10120; Invitrogen, Carlsbad, California). Jka or Jkb positive RBCs were enumerated by flow cytometry as previously described.[13]

Data Handling and Statistical Analyses

Statistical analysis of tracking of RCS was performed using linear mixed modeling. The data used for the RCS tracking of the adult and neonate BioRBC populations and the 2 Kidd antigen-mismatched RBC populations were expressed as ratios relative to the sample closest to 24 hours after the transfusion. Because blood samples for RCS tracking of individual infants were not available at precisely the same time, the ratios at the individual time points were grouped into intervals as follows: 3 days to <7 days, and weekly intervals thereafter (eg, 7 to <14 days through 49 to <64 days). Multiple data points for a subject within a given interval were treated statistically as replicates. Because the ratios did not have a normal distribution, a log transformation was applied to the data before linear mixed model analysis.

Two mixed models were fitted. One compared RCS tracking of adult and neonate BioRBC densities. The other compared RCS tracking of Kidd antigens (Jka or Jkb) with the RCS tracking of adult and neonate BioRBC densities. The fixed effects in both models included RBC donor type