Feasibility of Autologous Cord Blood Cells for Infants with Hypoxic-Ischemic Encephalopathy

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Objective To assess feasibility and safety of providing autologous umbilical cord blood (UCB) cells to neonates with hypoxic-ischemic encephalopathy (HIE).

Study design We enrolled infants in the intensive care nursery who were cooled for HIE and had available UCB in an open-label study of non-cyropreserved autologous volume- and red blood cell-reduced UCB cells (up to 4 doses adjusted for volume and red blood cell content, 1-5 $\times$ 10^7 cells/dose). We recorded UCB collection and cell infusion characteristics, and pre- and post-infusion vital signs. As exploratory analyses, we compared cell recipients' hospital outcomes (mortality, oral feeds at discharge) and 1-year survival with Bayley Scales of Infant and Toddler Development, 3rd edition scores $\geq$85 in 3 domains (cognitive, language, and motor development) with cooled infants who did not have available cells.

Results Twenty-three infants were cooled and received cells. Median collection and infusion volumes were 36 and 4.3 mL. Vital signs including oxygen saturation were similar before and after infusions in the first 48 postnatal hours. Cell recipients and concurrent cooled infants had similar hospital outcomes. Thirteen of 18 (74%) cell recipients and 19 of 46 (41%) concurrent cooled infants with known 1-year outcomes survived with scores >85.

Conclusions Collection, preparation, and infusion of fresh autologous UCB cells for use in infants with HIE is feasible. A randomized double-blind study is needed. *(J Pediatr 2014;164:973-9)*.

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Moderate hypothermia improves outcomes for term and near-term infants born with moderate or severe hypoxic-ischemic encephalopathy (HIE), but in pivotal trials over 30% of cooled infants died or survived with impairment.[1-4] The high prevalence of poor outcome provides motivation to test additional interventions.[5] Hypothermia targets pathophysiology related to secondary energy failure, including excitatory neurotransmitter release and destructive apoptosis and 'continuum' cell death.[6,7] Additional interventions may focus on these mechanisms plus interactions between injury, injury response, and ongoing brain development.[8]

Nucleated umbilical cord blood (UCB) cells can differentiate in vitro into cells with characteristics of neurons, oligodendrocytes, astrocytes, and microglial cells.[9-11] UCB cells have been used successfully in thousands of allogeneic transplants for cancer and genetic disease, including in infants with Krabbe disease and Hurler syndrome.[12,13] Neonatal rodents injected with human UCB cells after hypoxic-ischemic injury have improved anatomic and neurobehavioral outcomes, most likely because of paracrine and trophic effects during the hours and days after injury, leading to speculation that UCB cells could be a useful adjunct intervention for human infants with HIE.[14-19]

We hypothesized that early infusion of autologous volume- and red blood cell (RBC)-reduced UCB cells in infants with HIE would, primarily via trophic and paracrine mechanisms, improve outcomes. To that end, we conducted a pilot feasibility and preliminary safety study of IV infusion of non-cryopreserved RBC- and volume-reduced autologous UCB cells in infants with moderate or severe HIE. Our objectives were to: (1) identify challenges to coor-

Bayley III	Bayley Scales of Infant and Toddler Development, 3rd edition	ICN	Intensive care nursery
CCBB	Carolinas Cord Blood Bank	IND	Investigational new drug
CP	Cerebral palsy	MFM	Maternal-fetal medicine
DMSO	Dimethyl sulfoxide	NICHD	*Eunice Kennedy Shriver* National Institute of Child Health and Human Development
DUH	Duke University hospital		
DUHS	Duke University Health System	RBC	Red blood cell
FDA	Food and Drug Administration	SCL	Stem Cell Laboratory
HIE	Hypoxic-ischemic encephalopathy	UCB	Umbilical cord blood

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dinating the multiple disciplines needed to collect, prepare, and infuse cells in the first postnatal days; (2) characterize quality of UCB collections in high risk deliveries; and (3) report the cell recipients' response to infusions and their clinical outcomes at hospital discharge and 1 year of age.

Methods

We initiated this pilot study in January 2009. Infants admitted to the Duke intensive care nursery (ICN) were eligible if they were ≥ 35 weeks gestation with HIE and met the ICN cooling criteria, which is based on the inclusion criteria used in the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Neonatal Research Network hypothermia trial.[2,20] Hypothermia criteria were met if infants had cord or first postnatal hour blood gas results with pH ≤ 7.0, or base deficit ≥ −16. If a blood gas in the first postnatal hour was unavailable, or if the cord or first postnatal hour blood gas pH was 7.01-7.15 or base deficit was between −10 and −15, infants were eligible if they also had a history of an acute perinatal event and either an Apgar score at 10 minutes of ≤ 5 or need for positive pressure ventilation initiated at birth and continued for ≥ 10 minutes. Infants meeting criteria were then examined in 6 domains: level of consciousness, level of spontaneous activity, tone, posture, primitive reflexes, and autonomic function. If abnormal in 3 of 6 domains, or if the infant had seizures, it was treated with hypothermia and eligible for the study if cells were available.

UCB collection for donation to the Carolinas Cord Blood Bank (CCBB) for public banking within the Duke University Health System (DUHS) is routinely performed by dedicated, trained UCB collection staff and is restricted to deliveries of mothers who have given prior written informed consent for collection and have healthy term babies. If a CCBB donor mother delivered a baby with signs of HIE, CCBB staff collected UCB utilizing standard procedures, and UCB was deferred from public banking and instead, utilized if the sick infant was eligible for our study and the parents consented for study participation. For deliveries in which prior CCBB collection consent had not been obtained, the DUHS institutional review board gave permission for obstetric staff to obtain verbal assent to collect UCB if in the perinatal period the obstetric caregiver thought the infant could meet HIE cooling criteria. If cells were available, and the infant met cooling criteria, parents were asked to provide written informed consent for the infant to be enrolled in the study. The study was approved by the Duke institutional review board.

Cord blood was collected aseptically via in utero or ex utero techniques into cord blood collection bags (Pall, Medsep, Covina, California) containing 35 mL of citrate phosphate dextrose anticoagulant provided by the CCBB.[21] UCB collections were made by trained obstetricians, midwives, or CCBB collection staff. Collection staff were present at both DUHS Birthing Centers (Duke University Hospital [DUH], and Duke Regional Hospital, an affiliated community hospital approximately 5 miles from DUH) during weekdays for 8-12 hours per day. UCB collectors were also on site at 6 other regional centers not affiliated with the DUHS. UCB collected for outborn infants was sent with the infant on transport.

Collected UCB was transported at room temperature in validated shippers to the Duke Stem Cell Laboratory (SCL). There it was volume- and RBC-reduced after 20-30 minute incubation with 6% Hespan (hetastarch; Hospira, Lake Forest, Illinois) following established CCBB procedures using the Sepax 1 automated processing system (Biosafe, Geneva, Switzerland) if the unit contained >30 mL of UCB or manually if the unit was <30 mL. Volume- and RBC-reduced UCB cells were deposited into a volume of 20.5 mL and aliquotted into individual dose syringes with primed connecting tubing and a syringe containing a normal saline flush. A specialized storage bag with 4 needleless injection ports for removing sterile aliquots for up to 4 doses was developed for use in this trial by Biosafe. Total nucleated cell counts pre- and post-processing, post-processing $CD34^+$ cell content, colony forming units, sterility, and viability were assessed. Doses were engineered to contain 1-5 $\times 10^7$ nucleated cells, with no more than 2 mL/kg of packed RBCs and a targeted dose volume ≤2 mL/kg/dose.

All infants were cooled to 33.5°C for 72 hours.[2,20] Study staff carried premeasured doses of cells to the ICN in labeled syringes from the Duke SCL. Infusions were started when cells and study staff were available for administration and monitoring. Infants were pretreated with hydrocortisone, 1 mg/kg IV 30-60 minutes prior to infusion. Infants received up to 4 infusions of 1-5 $\times 10^7$ cells/kg, with the first dose as soon as possible after birth, and at 24, 48, and 72 postnatal hours. If the first dose was available after the first 12 postnatal hours, dose timing was adjusted to provide 3 infusions during the first 72 postnatal hours. After release of final guidance for public cord blood banking in 2011, the Food and Drug Administration (FDA) implemented a requirement for regulation of all non-homologous uses of UCB. Accordingly, we submitted an investigational new drug (IND) application for this study. With FDA approval of our IND application in July 2011, the protocol was modified to provide a maximum of 2 infusions of fresh cells in the first 48 postnatal hours. All infusions were administered in the Duke ICN. Infusate and subject identities were double-checked by research and clinical nursing staff. Infusions were monitored by research and clinical staff. Cells were infused over 15-20 minutes, followed by a 1-2 mL saline flush to clear the intravascular line. Unused cells were cryopreserved in the SCL after addition of 10% dimethyl sulfoxide (DMSO) to unused cells in a Medsep dual compartment cryopreservation bag (Pall Medical, Covina, California). Cells were cryopreserved after controlled-rate freezing under liquid nitrogen in a Thermogenesis bioarchive (Thermogenesis, Sacramento, California) for long-term storage.

Statistical Analyses

We used descriptive statistics to characterize subject demographics and feasibility measures, which included volume of UCB collected, time between collection, preparation and