Short Report

Feasibility of cord blood collection for autologous cell therapy applications in extremely preterm infants

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A B S T R A C T

Background aims: Umbilical cord blood (UCB)-derived cells show strong promise as a treatment for neonatal brain injury in pre-clinical models and early-phase clinical trials. Feasibility of UCB collection and autologous administration is reported for term infants, but data are limited for preterm infants. Here the authors assessed the feasibility of UCB-derived cell collection for autologous use in extremely preterm infants born at less than 28 weeks, a population with a high incidence of brain injury and subsequent neurodisability.

Methods: In a prospective study at a tertiary hospital in Melbourne, Australia, UCB was collected from infants born at less than 28 weeks and processed to obtain total nucleated cells (TNCs), CD34+ cells, mononuclear cells and cell viability via fluorescence-activated cell sorting prior to cryopreservation. Feasibility was pre-defined as volume adequate for cryopreservation (> 9 mL UCB collected) and > 25 × 10^6 TNCs/kg retrieved.

Results: Thirty-eight infants (21 male, 17 female) were included in the study. Twenty-four (63.1%) were delivered via cesarean section, 30 (78.9%) received delayed cord clamping before collection and 11 (28.9%) were a multiple birth. Median (interquartile range [IQR]) gestational age was 26.0 weeks (24.5–27.5) and mean (standard deviation) birth weight was 761.5 g (± 221.5). Median (IQR) UCB volume collected was 19.1 mL/kg (10.5–23.5), median (IQR) TNC count was 105.2 × 10^6/kg (57.4–174.4), median (IQR) CD34+ cell count was 1.5 × 10^6/kg (0.6–2.1) and median (IQR) cell viability pre-cryopreservation was 95% (92.1–96.0). Feasibility of collection volume and cell count suitable for cell cryopreservation was achieved in 27 (71%) and 28 (73.6%) infants, respectively.

Conclusions: UCB-derived cell collection adequate for cryopreservation and subsequent autologous reinfusion was achieved in 70% of extremely preterm infants. Extremely preterm UCB demonstrated a higher CD34+:TNC ratio compared with published full-term values. Recruitment to demonstrate safety of UCB cell administration in extremely premature infants is ongoing in the CORD-SAFE study (trial registration no. ACTRN12619001637134).

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Introduction

Preterm birth represents a primary cause of childhood morbidity and mortality worldwide [1], with brain injury a leading cause of neurodisability in survivors of extreme premature birth (less than 28 weeks) [2]. Targeted therapies for neonatal morbidities such as preterm brain injury remain limited, which has led researchers to investigate cell therapies such as those derived from cord blood as a potential therapeutic intervention. Umbilical cord blood (UCB) has an 

established role in hematopoietic stem cell transplantation and was first used for Fanconi anemia in 1988 [3]. It is now widely collected and stored for future use, with over 100 cord blood banks worldwide and greater than 4 million units stored to date [4]. In addition to hematopoietic stem cell transplantation, UCB-derived cell therapies are gaining increasing attention in the field of regenerative medicine. Rather than engraftment, UCB cells used for regenerative medicine applications act to exert paracrine and immunomodulatory effects—in particular anti-inflammatory, anti-apoptotic and pro-angiogenic effects—without engraftment [5–7]. These effects are exerted by the range of stem and progenitor cell types that comprise the mononuclear cells within a UCB unit, including hematopoietic stem and progenitor cells, regulatory T cells,
endothelial progenitor cells and mesenchymal stromal cells [8]. It is likely because of the aforementioned anti-inflammatory, pro-angiogenic and anti-apoptotic properties that UCB cells have shown promise in a broad range of pre-clinical models in neonatal medicine, such as hypoxia--ischemia [9,10] and pre-term brain inflammation [11], which are now being translated into early-phase clinical trials [12,13]. In a systematic review of UCB- and cord tissue-derived cell therapy for neonatal morbidities, 12 published early-phase clinical trials were identified and 24 ongoing clinical trials using these cell therapies in the neonatal population were listed as actively recruiting [14]. In just over half of these trials, the cell product administered is autologous UCB-derived mononuclear cells.

Although UCB collection and storage are well established in term infants, yielding on average 81 mL per collection [15,16], there are limited data on UCB volumes and cell yields in infants born at less than 32 weeks’ gestation. A German study of 141 UCB collections from preterm and term infants (23–41 weeks gestational age) showed a median UCB volume collected of 24.5 mL from a population with a median gestational age of 32.7 weeks and birth weight of 1635 g [17]. This study noted a correlation between birth weight and volume of UCB collected, and the researchers were able to successfully collect UCB in 80% of attempted cases. Another study from China assessed UCB collection after delayed cord clamping in preterm infants born at less than 35 weeks, with researchers conducting 41 collections from a population with a mean (standard deviation) gestational age of 31 weeks (2) and mean birth weight of 1642 g [18]. The researchers were able to collect an average of 47 mL of UCB per collection and were successful in 81% of attempted collections. There have been no reported studies specifically examining UCB collection for extremely preterm infants born at less than 28 weeks. In the present study, the authors aimed to investigate whether UCB-derived cell collection is feasible for autologous reinfusion to the extremely preterm population (less than 28 weeks gestational age), the neonatal cohort with the highest risk of morbidity and mortality, thus warranting future exploration of cell therapies.

Methods

Design

A single-center prospective study was conducted with informed antenatal parental consent prior to active labor at a tertiary maternity hospital in Melbourne, Australia. UCB collections were approved by the Monash Health Human Research Ethics Committee, first under UCB and stem cell collection ethics approval (human research ethics committee reference no. 12387B) and subsequently under human research ethics approval for the CORDSAFE study (trial registration no. ACTRN12619001637134, human research ethics committee reference no. RES-19-0000-632A) [19]. Eligible infants who were included in the study were those born at less than 28 weeks gestational age for whom active management was planned (23 + 0 to 27 + 6 weeks) and for whom antenatal consent was given. Infants with known major congenital malformations and those for whom parents were unable or unwilling to provide informed consent (e.g., because of emergent delivery) were excluded.

UCB collection

UCB was collected at both vaginal and cesarean section deliveries. Where possible, UCB was collected using in utero collection, where UCB was collected prior to separation of the placenta. A total of 90% of collections were performed by a neonatologist, with the remaining small minority collected by an obstetrician or trained cord blood collector. This involved a collector being scrubbed within the sterile field at cesarean section deliveries. UCB was collected using an 18-gauge needle and 20-mL syringe, and blood was immediately placed into a standard UCB collection bag with anticoagulant (Macopharma, Tourcoing, France) and sent for processing. This method was adapted from standard term UCB collection procedures in which a larger-bore needle is used but was found to be too large for insertion in some umbilical vessels in extremely pre-term infants. A maximum of three umbilical vein punctures were accepted per collection attempt. Processing was completed according to the cord blood unit processing procedure at Cell Care (Moorabbin, Australia), which includes partial red cell depletion, testing for microbiological contamination, blood group antigen testing and cell counts via fluorescence-activated cell sorting.

Outcomes

Feasibility was pre-defined as obtaining adequate UCB volume for cryopreservation (> 9 mL) and at least 25 × 10⁶ total nucleated cells (TNCs) per kg infant body weight. Demographic data were collected from maternal and infant electronic medical records and UCB volume and cell counts recorded.

Statistical analysis

Descriptive statistics were used to describe demographic data and outcomes. A Shapiro–Wilks test was used to determine normality of data. Categorical variables were reported as frequency and percentage, and continuous data as median (interquartile range) and mean (standard deviation). Pearson r was used to examine the relationship between gestational age and birth weight and UCB volume collected, with significance determined at P < 0.05. Data analysis was completed using Prism 9.3 (GraphPad Software, San Diego, CA, USA).

Results

Demographics

Thirty-eight infants (21 male, 17 female) were included in the study. Twenty-four (63.1%) were delivered via cesarean section, 30 (78.9%) received at least 60 s of delayed cord clamping prior to UCB collection and 11 (28.9%) were a multiple birth. Median (interquartile range [IQR]) gestational age was 26 weeks (24.5–27.5) and mean (standard deviation) birth weight was 761.5 g (221.5). Demographic data are displayed in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n</td>
<td>38</td>
</tr>
<tr>
<td>Gestational age at birth, weeks, median (IQR)</td>
<td>26.0 (24.5–27.5)</td>
</tr>
<tr>
<td>Birth weight, g, mean (SD)</td>
<td>761.5 (221.5)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>21 (55.2)</td>
</tr>
<tr>
<td>Reason for delivery, n (%)</td>
<td></td>
</tr>
<tr>
<td>Preterm labor</td>
<td>26 (68.4)</td>
</tr>
<tr>
<td>Antepartum hemorrhage</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>Fetal growth restriction</td>
<td>4 (10.5)</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>5 (13.1)</td>
</tr>
<tr>
<td>Mode of delivery, n (%)</td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>24 (63.1)</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>14 (36.9)</td>
</tr>
<tr>
<td>Multiple births, n (%)</td>
<td>11 (28.9)</td>
</tr>
<tr>
<td>Delayed cord clamping, 60 s, n (%)</td>
<td>30 (78.9)</td>
</tr>
</tbody>
</table>

SD, standard deviation.
The median (IQR) TNC count was 105.2 $\times 10^6$/kg (57.4–174.4) and median (IQR) cell viability was 95% (92.1–96.0). The maximum UCB volume collected was 43 mL and the maximum TNC count obtained was 1023.9 $\times 10^6$/kg. The median (IQR) CD34+ cell count was 1.5 $\times 10^6$/kg (0.6–2.1) and the median (IQR) CD34+ percentage of TNCs was 1.1% (0.7–1.7). The median (IQR) mononuclear cell count was 42.8 $\times 10^3$/kg (24.1–106.1). For the five infants born in the context of clinical chorioamnionitis, TNC counts were significantly higher than the rest of the cohort, with an average TNC count of 359 $\times 10^6$/kg (range, 147–1023). Two samples were affected by microbiological contamination, both also in the context of chorioamnionitis. The authors did not find a positive correlation between gestational age at birth and either TNC count or CD34+ cell count. Feasibility of collection volume (9 mL) and cell count suitable for cryopreservation and future reinfusion (25 $\times 10^6$/kg) was achieved in 27 (71%) and 28 (73.6%) infants, respectively. UCB volume, cell count and feasibility data are displayed in Table 2.

Discussion

To the authors’ knowledge, this is the first study to specifically report the feasibility of UCB cell collection in extremely preterm infants born at less than 28 weeks’ gestation, representing the most immature cohort in whom UCB collection has been studied. The authors demonstrate that it is feasible to collect an adequate UCB volume and cell count for potential autologous use in approximately 70% of infants in this population. In addition, the authors conducted this study in a setting where delayed cord clamping become routine for pre-term deliveries, with 78.5% of collections occurring after 60 s of delayed cord clamping.

UCB was successfully collected in 90.5% of the infants studied, which is slightly higher than that noted in previously reported studies (80% and 81%) [17,18], but the overall success rate for achieving the authors’ feasibility outcome (> 9 mL) for cryopreservation was 71%. If future study protocols do not require cryopreservation, some infants are still likely to have > 25 $\times 10^6$ TNCs/kg in a collection volume of less than 9 mL. The authors have confirmed previous findings of a correlation between birth weight, gestational age and UCB volume collected, which has been demonstrated in term and older pre-term infants [17,20], but this is the first published report in an extreme preterm population.

A previous study of 33 preterm infants (median, 32 weeks) demonstrated a higher proportion of hematopoietic progenitor (CD34+) cells in preterm cord blood compared with term controls [21], and the authors have confirmed these findings in the present study in an extremely preterm population. The infants in the authors’ study had an average CD34+ : TNC ratio of 1.1% (with one infant as high as 3.6%), which is very high in comparison with a median CD34+ : TNC ratio of 0.34% for term UCB in the US inventory [22]. Differences such as these in the composition of pre-term UCB may have implications for future cell therapy applications, with a potentially higher stem and progenitor cell yield from lower collected volumes.

There are several factors the authors suggest would have an impact on the success of UCB collection for extremely preterm infants. These include timing of delivery, experience of the collector and underlying pathology causing progression to pre-term delivery. In the authors’ study, most collections were performed by a neonatologist involved in the project. This is not part of routine practice for a neonatologist, and it may be that other experienced professionals, such as obstetricians and midwives, would be more proficient at UCB collection. Notably, the largest collected volume in the study was obtained by an experienced obstetrician. Time of day of delivery also has an affect on logistical success. A total of 70% of infants in this study were born outside of normal business hours, and in three cases involving consented individuals, research staff were unable to attend within the required time frame. This could potentially be mitigated by educating and training a broader range of staff in performing UCB collection if it becomes a more widespread practice in preterm deliveries.

Although the numbers are small, this study has identified pathological factors that may lead to low UCB yield, in particular birth in the context of antepartum hemorrhage, placental abruption and severe fetal growth restriction. With regard to the seven infants born...
under these circumstances, none yielded adequate UCB volume for cryopreservation (≤9 mL obtained), whereas one yielded an adequate cell count for autologous use (UCB volume 7 mL, cell count 323.5 × 10^6 /kg). For infants such as these, where UCB collection is not successful or of low volume, alternative cell therapies, such as expansion of the cord blood that can be collected, may need to be explored.

This study is being undertaken with the aim of developing UCB-derived cells as a neuroprotective therapy. In the CORD-SAFE study [19], two doses are being trialed (25 × 10^6 cells/kg and 50 × 10^6 cells/kg) based on pre-clinical data demonstrating that 50 × 10^6 cells/kg is likely to be a neuroprotective dose [23,24]. In addition, there are pre-clinical data to suggest that multiple doses may be more efficacious than one [25], and a multiple-dose approach has now been tested in phase 1 clinical trials of UCB-derived cells in newborns [12,26]. However, as the present data demonstrate, some infants may not have adequate autologous cells for a high- or multiple-dose treatment protocol, which is where UCB cell expansion may be of use.

UCB-derived cells have been successfully expanded ex vivo [27] and in sufficient quantities to be administered for the purpose of hematopoietic stem cell transplantation [28]. It is thus likely that expansion of UCB cells from low-yield collections would be possible, augmenting the cell dose available to allow higher-dose or multiple autologous cell infusions if the therapy is shown to be safe and effective. Expanded umbilical cord-derived mesenchymal stromal cells are already being used in neonatal clinical trials [29,30], and allogeneic UCB cell therapy has been administered for neurological conditions and in children with cerebral palsy [31,32]. These may also be alternative UCB-derived cell therapy solutions for those infants who do not have adequate UCB cells collected for autologous therapy.

Limitations

This was a single-center study to assess the feasibility of UCB collection from extremely preterm infants. It was conducted in a well-resourced setting and with experienced research staff available to assist. The results may not be applicable to the birth of extremely preterm infants in low-resource settings. There were relatively few infants born with antenatal complications of antepartum hemorrhage and fetal growth restriction (n = 7), making it difficult to comment on their effect on UCB collection.

Conclusions

UCB-derived cell collection is feasible for approximately 70% of extremely preterm infants born at less than 28 weeks' gestation. Safety of an autologous UCB-derived cell therapy needs to be assessed in this population, and more research on alternative cell therapies is needed for the approximately one third of these infants who may not have adequate cells available for autologous therapy.

Declaration of Competing Interest

GJ is a member of the scientific advisory board of Generate Life Sciences (Los Angeles, CA, USA).

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Author Contributions

Conception and design of the study: SLM and AM. Acquisition of data: LZ, TP. Analysis and interpretation of data: LZ, CM, TY. Drafting or revising the manuscript: LZ. All authors have approved the final article.

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References


