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From cord to caudate: characterizing umbilical cord blood stem cells and their paracrine interactions with the injured brain

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Stem cells are proving to be a promising therapy for a wide range of pediatric disorders, from neonatal hypoxic–ischemic encephalopathy to pediatric leukemia. Owing to their low immunogenicity and ease of availability, umbilical cord blood (UCB) progenitor cells are increasingly replacing fetal- and adult-derived cells in therapeutic settings. Multiple environmental and demographic factors affect the number and type of stem cells extracted from UCB, and these differences have been associated with disparities in outcomes after transplantation. To avoid variations in efficacy, as well as the potential adverse effects of stem cell transplantation, evaluation of the stem cell secretome is critical to identify key paracrine signals released by the stem cells that could be used to provide similar neuroprotective effects to stem cell transplantation. This article describes the cell types found in UCB and reviews the available literature surrounding the effects of collection timing and volume, maternal risk factors, delivery characteristics, and neonatal demographics on the cellular composition of UCB. In addition, the current findings regarding the stem cell secretome are discussed to identify factors that could be used to supplement or replace stem cell transplantation in pediatric neuroprotection.

Stem cells have demonstrated significant promise in preclinical studies of neuronal repair in pediatric disorders such as hypoxic–ischemic encephalopathy (HIE), stroke, intraventricular hemorrhage, and traumatic brain injury (1,2). In addition, early clinical studies have shown both clinical feasibility for administration of stem cells after neonatal HIE and efficacy in decreasing cerebral inflammation in pediatric patients with cerebral palsy (3,4). Umbilical cord blood (UCB) is an attractive source of stem cells, since its collection is noninvasive, painless, and does not evoke the ethical concerns of embryonic stem cells. Although the number of nucleated cells recovered from UCB is significantly lower than that from bone marrow (BM), there is a higher frequency of primitive cells in UCB. The immature T-lymphocytes contained in UCB suppress the activation of lymphocytes and natural killer cells, resulting in low immunogenicity and pathogenicity (5,6). The ease of collection combined with the increased proliferative capacity of the more primitive cells compensate for the potential difficulty obtaining adequate numbers of nucleated cells, supporting the use of UCB as a valuable source of stem cells for therapeutic interventions.

To attempt to overcome the paucity of nucleated cells in UCB, one must understand not only the conditions that affect the numbers and quality of stem cells in UCB but also the neurotrophic and growth factors involved in stem cell–mediated repair that can potentially be upregulated through genetic manipulation of the cells. These factors can enhance the efficacy of stem cells if delivered together or may act as a replacement for cell transplantation. This review will discuss the conditions affecting UCB stem cell numbers and quality, as well as the stem cell secretome and its interactions with the injured brain.

STEM CELL TYPES DERIVED FROM THE UMBILICAL CORD AND PLACENTA

UCB is not the only source of UC–derived stem cells; other UC–derived populations such as UC perivascular cells, umbilical vein endothelial cells, and UC–lining stem cells have also been suggested as alternatives to UCB (***Figure 1***). Compared with UCB, higher numbers of mesenchymal stromal/stem cells (MSCs) can reliably be extracted from the various compartments of the UC. One of the most readily accessible UC stem cell sources is the Wharton's jelly. Wharton's jelly is the mucous connective tissue between the amnion and the umbilical vessels and contains a native population of MSCs referred to as Wharton's jelly stem cells (7). Wharton's jelly stem cells demonstrate a higher frequency of colony-forming unit fibroblasts than either UCB MSCs or BM MSCs (8), and like UCB MSCs, Wharton's jelly stem cell MSCs are multipotent (9). In addition to UC tissue, epithelial and mesenchymal cell types may also be derived from the amnion and chorion of the placenta (10). This review will focus primarily on UCB, as most of the UC–derived stem cell literature to date has focused primarily on UCB. It is important, however, to understand that other stem cell populations extracted from the UC and placenta are also

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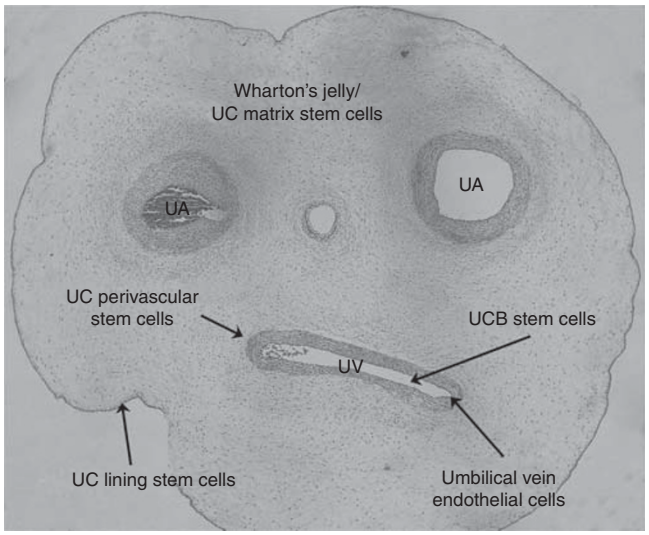

Figure 1. Sections of the umbilical cord that may be harvested for stem cells. UA, umbilical artery; UC, umbilical cord; UCB, umbilical cord blood; UV, umbilical vein. Figure adapted and modified from (ref. 97) © 2016 Uthman CC-BY, version 2.0.

being examined for their potential therapeutic effect in pediatric brain injury, and we may discover that UCB MSCs are less effective than one or more of the other UC–derived populations.

UCB contains numerous stem and progenitor cell types, including hematopoietic stem cells, MSCs, and the unipotent endothelial progenitor cells (***Figure 2***). MSCs, however, are the only UCB–derived stem cell population with the potential for neuronal differentiation, and as such, they will be the primary focus of this review. MSCs are multipotent nonhematopoietic cells defined by the International Society for Cellular Therapy as having $\geq 95\%$ expression of CD105, CD73, and CD90, and $\leq 2\%$ expression of CD45, CD34, CD14, CD11b, CD79a, or CD19, and HLA-DR surface molecules. Additionally, an MSC must be able to differentiate into osteoblasts, chondroblasts, and adipocytes *in vitro* (11).

A unique subset of MSCs has been identified in UCB, which express pluripotent stem cell markers and are capable of differentiating into neurons after exposure to simple induction conditions *in vitro* (12). Other UCB MSCs have been induced *in vitro* to express neural-specific antigens and take on the morphology of neural cells after being exposed to neural culture (13). In support of this differential capability, some studies have reported migration and differentiation of UCB mononuclear cells into astrocytes when transplanted either intraperitoneally or intracerebrally in a rodent model of neonatal hypoxic–ischemic brain injury (14–16). Compared with BM or UC cells, UCB contains lower number of MSCs (17,18), making it increasingly important to understand the factors that may increase the numbers or potency of MSCs in UCB to maximize their therapeutic potential.

EFFECT OF COLLECTION FACTORS ON UCB

Collection factors such as increased blood volume and decreased time to isolation are two of the primary drivers in successful isolation of stem cells from UCB (**Table 1**). Al-Qahtani *et al.* (19) demonstrated that collection volume was the most significant factor among 16 maternal, neonatal, and obstetric variables in predicting the number of total nucleated cells (TNCs) derived from UCB samples (19). The few studies that have assessed these factors in greater detail suggest that a net volume >33–80 ml and time from collection to isolation <10–15 h significantly increase the yield of CD34+ cells from UCB (20,21). Because of the strong association between cord blood volume and stem cell yield, the increasing frequency of delayed cord clamping (DCC) has been closely analyzed due to the concern that it could decrease the volume of blood remaining in the placenta and UCB.

Two studies evaluating the effects of DCC on UCB characteristics have found lower TNC counts in DCC compared with immediate cord clamping, with 17–37% of samples meeting a TNC threshold of 15×10^8 cells for eligibility for cord banking in the DCC group and 37–47% in the immediate cord clamping group (22,23). The difference was no longer significant at a threshold of 12.5×10^8 cells, however, with 52% of the DCC group vs. 60% in the immediate cord clamping group meeting criteria (23). Despite the significant difference between groups, the authors highlighted that many cord blood units still met the highest threshold despite DCC, implying that collection of quality UCB stem cells continues to be feasible despite DCC. In addition, the clinical benefit of DCC likely outweighs the potential for lower stem cell yield. Not only has DCC been associated with decreases in intraventricular hemorrhage and respiratory distress syndrome, but a number of authors have argued that DCC may be the most effective way to noninvasively administer autologous stem cells (24–26).

Cord milking is another procedure that has been used to improve passage of placental blood to the neonate immediately after birth, especially in situations where DCC may be inappropriate due to the need for resuscitation (27). Although one study demonstrated higher systemic blood flow after cord milking compared with DCC in preterm infants (28), there does not appear to be a significant difference in the amount of blood transferred between the two procedures (29), and the effect of cord milking on UCB collection has not yet been thoroughly evaluated.

EFFECT OF FETAL STRESS ON UCB

When exposed to certain environmental factors such as acute hypoxia, stem cells demonstrate improved proliferation and function. UCB MSCs cultured under hypoxic conditions display increased hypoxia-inducible factor-1α expression, angiogenic cytokines, and improved proliferative capacity *in vitro*. Transplantation of hypoxia-preconditioned stem cells into a mouse model of an ischemic limb resulted in decreased muscle atrophy, bone loss, and apoptosis, and increased

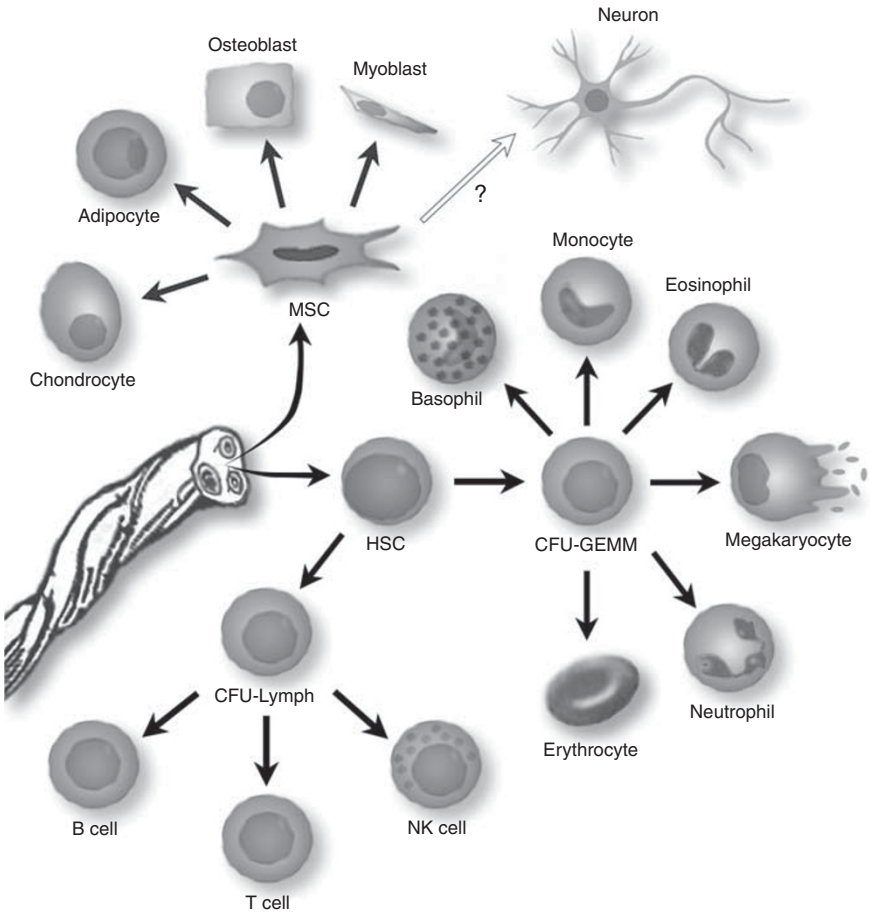

Figure 2. Potential differentiation of mesenchymal (MSC) and hematopoietic stem cells (HSC) derived from the umbilical cord and umbilical cord blood. CFU, colony-forming unit; GEMM, granulocyte, erythroid, macrophage, and megakaryocyte.

Table 1. Summary of the effects of maternal and neonatal factors on umbilical cord blood characteristics

	Maternal factors			Neonatal factors							
	Weight	Parity	Age	BW	GA	Placental weight	UC length	Sex		Race[a]	
								M	F	Caucasian	Black
UCB volume				++	+	+	+	++	+		−
CFU		−		++	−	+	+	++	+	+	
CD34+		−		++	−−	+	+	++	+	+	
TNC count	+	−		++	+	+	+	+	++		

Abbreviations: BW, birth weight; CFU, colony-forming units; GA, gestational age; TNC, total nucleated cells; UC, umbilical cord; UCB, umbilical cord blood.
Empty cells represent associations without adequate data.
[a]Listed effects representative of listed race vs. other races. Other races were not included due to inadequate data.

capillary and arteriole density correlating with the level of hypoxic preconditioning (30,31).

The effects of hypoxia on MSC function have not been well evaluated clinically. Studies of UCB endothelial colony-forming cells (ECFC), however, have assessed the effects of chronic hypoxia by investigating fetuses exposed to placental insufficiency caused by maternal diabetes mellitus or preeclampsia. As opposed to the improved proliferation and function of MSCs after acute *in vitro* hypoxia, UCB ECFC experiencing chronic hypoxia secondary to maternal diabetes tend to exhibit decreased vessel formation, increased senescence, and decreased proliferation, especially after multiple passages (32,33). This may be in part due to a difference in the ability to express vascular endothelial growth factor. UCB ECFCs obtained from patients from nondiabetic pregnancies demonstrated increased vascular endothelial growth factor-A expression when exposed *in vitro* to moderate hypoxia for 72 h. ECFCs obtained from pregnancies affected by gestational diabetes showed no increase in vascular endothelial growth factor-A expression after hypoxia (34). In mothers with preeclampsia, the UCB volume, TNC count, CD34+ cell count, and the number of ECFCs in UCB are

significantly decreased, and the cells are slower to emerge in culture compared with controls (35,36). Taken together, these findings suggest that controlled acute hypoxia may help to increase the angiogenic function of UCB stem cells *in vitro*, although conditions such as gestational diabetes or pre-eclampsia that may cause chronic hypoxia *in vivo* tend to result in decreased ECFC angiogenic function.

EFFECT OF MATERNAL FACTORS ON UCB

In addition to maternal diseases such as preeclampsia and diabetes mellitus, other pregnancy complications and lifestyle choices can influence the quality and quantity of cord blood. For instance, although preeclampsia has an overall negative effect on the volume and cell count of UCB, maternal hypertension without preeclampsia has been shown to produce UCB with higher CD34+ cell counts (37). Maternal smoking has no documented effect on the quality of UCB, but has been demonstrated to result in lower UCB volume than in nonsmoking pregnancies (38). It is unclear if this is due directly to smoking or is secondary to smoking's effects on fetal growth, resulting in lower birth weight.

Other maternal factors such as maternal body habitus and parity have also been associated with cord blood quality. Higher maternal weight is associated with an increased TNC count (37,39), although no association was found with maternal height. In addition, lower parity is positively correlated with TNC, colony-forming units (CFU), and CD34+ counts (39,40), and with each additional previous birth there is a 17% decrease in CD34+ counts (38). The influence of other factors such as maternal age is still poorly understood. Although some studies have found that younger maternal age leads to higher cell counts in UCB, others have found that mothers 20 years or older have higher CFU, CD34+, and TNC counts (21,41). Still, more studies have found no correlation between maternal age and quality of UCB (38).

EFFECT OF NEONATAL FACTORS ON UCB

The quantity and quality of UCB correlate with several neonatal factors, including birth weight, placental weight, and umbilical cord length. Studies have consistently demonstrated an association between higher birth weight and improved UCB quantity and quality, defined as a higher number of CFU, CD34+ cells, or TNC (21,38,42). It has been estimated that every 500 g increase in birthweight results in a 6% higher volume of UCB collected. The same increase in birthweight increases the CFU yield by 9–21%, CD34+ count by 11–28%, TNC count by 6–11%, and the ability to produce an acceptable UCB TNC count of 8×10^8 cells by 40% (21,38,43). In a multivariate model assessing an association between TNC count and 10 different maternal and neonatal factors, birth weight was the strongest predictor of TNC count (39). In addition to birth weight, placental weight and umbilical cord length are also positively correlated with the volume of UCB collected and the number of TNC, CD34+ cells, and CFU (40,41).

Longer gestation is correlated with a higher volume of UCB extracted and a higher TNC count. The cells extracted, however, tend to be more mature at higher gestational ages, demonstrating less CD34+ cells and lower CFU (39). For each additional week of gestational age, TNC count increases by 3% and CD34+ counts decrease by 9% (38). The increase in TNC count is thought to be due in part to increased placental size and weight at higher gestational ages. This balance between quantity and quality of UCB is important when considering the therapeutic potential of UCB. Preterm UCB contains fewer nucleated cells, but those that are extracted have increased proliferative capacity compared with term and post-term neonates (21,44). Conversely, term and post-term UCB contains significantly more nucleated cells, resulting in 46% more successful cord blood units collected, but the cells contained in the units are less potent (21,42).

The sex of the neonate may also influence the quantity and quality of the UCB obtained. Males tend to have higher cord blood volume, CFU, and CD34+ counts, whereas females have a higher TNC count (21,38,45–47). It is important to note, however, that many neonatal variables are highly correlated and may confound any observed univariate associations. For instance, there is a strong correlation between sex and birth weight, with male infants having higher birthweights on average than females. Thus, it is unclear if the relationship between sex and UCB characteristics is a true correlation or primarily due to the difference in birth weight. After correcting for birth weight, one study found that males have higher CD34+ count, CFU, and hematopoietic progenitor cell concentration (46), although another found that there was no association between sex and UCB quality (43). Further studies assessing the association between neonatal sex and UCB characteristics should include multivariate analyses to correct for birth weight.

One benefit of the ease of UCB collection is that it allows for increased potential for collection from underrepresented races and ethnicities; however, race may also influence the quality of the UCB collected. The effect of race on UCB quantity is controversial, as some studies have found similar collection volumes between all races, and others have found decreased volumes in African-American infants (40,48). Regardless of the volume, Caucasian neonates have been found to have the highest CD34+ counts and CFU, followed by multiracial, Hispanic, African American, and Asian neonates (21,45). Some studies have also found higher TNC counts in Caucasians, although others have found no significant difference between races (21,38,42). One of the greatest limitations to successfully answering the question of whether there is an association between race and UCB is that the current literature does not accurately represent the racial diversity present in most communities. Most studies rely on self-reported racial and ethnic demographics that often do not account for the race or ethnicity of the father, and almost universally do not account for multiracial backgrounds. Future studies using more detailed racial data or genetic

analyses will be necessary to truly understand the effects of race on the quality of UCB.

EFFECT OF DELIVERY FACTORS ON UCB

Although many studies have attempted to assess the relationship between mode of delivery and UCB, much of the data have been conflicting. One of the few consistent findings is that there tends to be a higher volume of UCB collected in cesarean deliveries vs. vaginal deliveries. The difference in volume may reflect the active manipulation of the placenta during a C-section as opposed to physiologic delivery of the placenta in vaginal deliveries, or potentially the difference in infant position; most vaginal deliveries result in the infant positioned lower than the placenta, allowing gravity to facilitate blood transfer from the placenta to the infant (41,49). Last, the controlled nature of the operating room may be why UCB collection has been found to be ~43% more successful after C-section compared with vaginal deliveries (42,43).

Similar inconsistencies have come from studies assessing TNC counts between modes of delivery. Some studies have demonstrated higher TNC counts and cell quality in vaginal deliveries, which has been cited as being due to the stress on the fetus that results from labor (37,48). Supporting that theory, C-section deliveries were found in one study to be 40% less likely than vaginal deliveries to have adequate TNC counts for clinical use (42,43). Several other studies, however, have demonstrated higher TNC counts in C-section deliveries, hypothesizing that the more rapid UC clamp time in Cesarean deliveries results in more blood in the placenta and umbilical cord, and therefore increased TNCs (42,49). A similar controversy exists regarding CFU and CD34+ cells, with some studies demonstrating higher total CFU or CD34+ cells in cesarean deliveries, and others showing no difference (37,45,46).

When comparing elective to emergent or urgent C-section, a higher volume of cord blood has been found with elective C-sections, although the quality of the cells appears to be improved in urgent or emergent C-sections. The higher volume collected in elective C-sections is thought to be primarily due to their scheduled nature, which allows for a more organized UCB collection process. Emergent or urgent C-sections lead to a higher TNC and CD34+ cells collected vs. scheduled C-sections (42,50), which may be due to the fetal distress that frequently leads to the emergent or urgent procedures.

One of the most important lessons that can be derived from the research on delivery mode and UCB collection is that the environment surrounding delivery is not binary. Limiting the description of the delivery experience to vaginal vs. cesarean delivery ignores many critical factors in UCB collection, including timing of cord clamping, placental manipulation, duration of labor, and the amount of fetal distress. With that in mind, it is worth noting that many of the studies cited here were performed before the widespread adoption of DCC practices.

THEORIZED MECHANISMS OF ACTION OF UCB IN BRAIN INJURY

In addition to selecting the most effective stem cell populations for transplantation, optimizing the clinical efficacy of stem cell transplantation in neurologic injury will require a thorough understanding of the underlying neuroprotective mechanisms of action of the UCB stem cells. With improved knowledge regarding stem cell signaling, we may also develop the ability to further select UCB cell subpopulations or alter cell expression to provide increased transplantation efficacy. With this in mind, the following section will discuss some key components of the stem cell secretome.

To date, there remains considerable debate regarding the mechanisms of action of the MSC's neuroprotective and neuroregenerative properties. Although stem cells have been shown to possess the capacity to develop into adult neurons, oligodendrocytes, and astrocytes *in vitro* and *in vivo* (51), it is now widely believed that they primarily function more distally through autocrine and paracrine mechanisms by the release of cytokines, growth factors, and other signaling molecules. This theory is supported by studies demonstrating decreased brain injury after treatment with cell-free MSC–conditioned media that is known to contain factors such as insulin-like growth factor-1 (IGF-1) and nerve growth factor (NGF) (52,53).

MSC transplantation results in the upregulation of two primary classes of molecules that are known to affect brain growth and development (***Figure 3***). The first class consists of the neurotrophins, which promote the survival and differentiation of neural stem cells and neuroprogenitor cells, and include brain-derived neurotrophic factor (BDNF) and glial cell–derived neurotrophic factor. BDNF and glial cell–derived neurotrophic factor are secreted by human BM–derived MSCs, and may be primary components of the neuroprotective effects of MSCs, as blocking their activity significantly attenuates the neuroprotective effect of

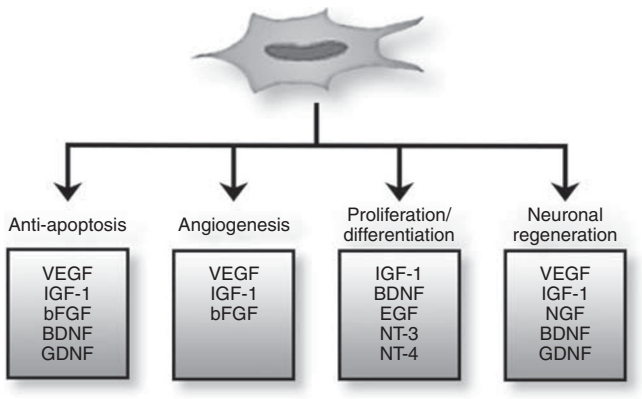

Figure 3. Examples of the neuroprotective actions of the stem cell secretome. bFGF, basic fibroblast growth factor; BDNF, brain-derived neurotrophic factor; EGF, epidermal growth factor; GDNF, glial cell-derived neurotrophic factor; IGF-1, insulin-like growth factor-1; NGF, nerve growth factor; NT-3, neurotrophin-3; NT-4, neurotrophin-4; VEGF, vascular endothelial growth factor.

stem cell–conditioned media (54,55). In a neonatal rat model of HIE, intracerebral BDNF administration significantly decreases caspase-3 activation and tissue loss (56,57). Similarly, NGF is a neurotrophin that aids in the survival and differentiation of neurons and has been shown to be expressed at higher levels after MSC transplantation, both by the host tissue as well as the grafted MSCs (58–60). The only published report of NGF administration in human neonates demonstrated clinical improvement in electroencephalography, cerebral perfusion, and a marker neurogenesis in two infants who received intraventricular injections of NGF after hypoxic–ischemic injury (61).

The second class of molecules upregulated by MSC transplantation consists of the neuroprotective cytokines, including IGF-1. IGF-1 is a growth factor that has been detected in BM MSC–conditioned medium (62), and has been demonstrated to improve BM MSC survival and proliferation during early differentiation (63,64). Inhibiting IGF-1 activity in a model of cerebral infarction resulted in a significant decrease in neural progenitor proliferation (65). In a neonatal animal model, intranasal or intracranial IGF-1 increased progenitor cell proliferation in the subventricular zone, attenuated brain injury, and improved neurobehavioral performance when administered after induced hypoxic–ischemic brain injury (66,67).

Understanding these molecular factors and their effects may allow investigators in the future to modify stem cells before transplantation to up- or downregulate certain factors to optimize therapeutic effects.

STATUS OF CLINICAL ADMINISTRATION OF UCB FOR PEDIATRIC BRAIN INJURY

Some of the earliest clinical studies of stem cell administration for nonhematological disorders were performed on infants and children with metabolic disorders. A study in 2004 demonstrated feasibility and favorable outcomes after UCB transplantation in children with Hurler's disease (68), which was followed shortly by two additional studies detailing the administration of UCB to infants with Krabbe's disease (69) and several other lysosomal and peroxisomal storage disorders (70). With these studies demonstrating feasibility and relative safety, and the mounting preclinical literature, investigators have begun to evaluate stem cell administration for a wide range of neonatal and pediatric brain disorders.

Although they are some of the best-studied acute neurological diseases of childhood in the preclinical literature, HIE and stroke have only just begun to produce early clinical trials of stem cell transplantation. One clinical study has been performed in infants after HIE which demonstrated clinical feasibility as well as early positive benefits with improved developmental outcomes at 1 year of life (4). The same investigators are currently undertaking a phase II randomized controlled trial of UCB in HIE, using survival at 1 year and Bayley Scales of Infant Development ≥ 85 as their primary outcome measures. There may be a surge of data in this field in the near future, as there are currently seven other clinical trials registered with Clinicaltrials.gov, two of which are randomized controlled trials.

Many of the other perinatal and neonatal brain injuries have even less clinical data to support UCB transplantation. A recent study described a case report of UCB administered 4 years after a perinatal stroke that resulted in significant improvement in the subject's motor function (71). No clinical trials in perinatal stroke have been performed, however, and only one is currently ongoing, but has suspended recruitment. Similarly, phase I and phase II trials are in progress to evaluate the benefits of a commercial stem cell product on intraventricular hemorrhage in preterm infants, but no results have been published. Last, feasibility and safety of UCB transplantation have been assessed in congenital hydrocephalus (72), but no studies have evaluated outcomes in this population.

Chronic pediatric brain disorders generate added questions regarding timing of UCB administration and appropriate number of repeated transplantations. Nevertheless, several clinical trials have begun to evaluate the use of UCB stem cells in chronic pediatric disorders such as cerebral palsy (3,73–81). Although several of these studies have included only a few patients, as a whole they contain over 600 subjects and demonstrate feasibility and safety of UCB administration in this population. Thorough evaluation of efficacy and the development of a standardized administration protocol for these patients, however, is still needed, and will hopefully be provided by several of the clinical trials that are currently in progress, including two here in the United States.

LIMITATIONS TO THE CLINICAL USE OF STEM CELLS FOR PEDIATRIC BRAIN INJURY

One question that must be answered before clinical implementation of stem cells for brain injury is the ideal route of administration. Several different routes have been trialed in preclinical studies using stem cell transplantation for brain injury. These include intraventricular, intraperitoneal, intravenous, intrathecal, intranasal, and intraparenchymal injections into the cerebral cortex (82–89). One study examined the difference between intravenous and intraparenchymal transplantation of UCB MSCs in treating cerebral hypoxic injury. They found that intravenous administration resulted in better long-term outcomes than intraparenchymal, although both had better developmental recovery than non-transplanted animals (82). Similarly, a study using Wharton's jelly stem cells in a rat model of HIE demonstrated that intravenous administration resulted in improved behavioral outcomes and decreased gliosis in the areas of injury. Intraperitoneal transplantation in the same population exhibited less improvement compared with intravenous (86). Intravenous administration therefore appears to be the least invasive and most effective mode of administration. Recently, however, studies have begun to assess the efficacy of intranasal administration, which has shown to be safe and effective in neonatal brain injury (84,90–92). No comparison studies between intravenous and intranasal administration in

pediatric brain injury have been reported. For now, clinical trials have been limited to intravenous, intraventricular, and intrathecal administration, and have not yet evaluated the intranasal route (4,81,83).

As with all therapies, it is important to thoroughly evaluate potential short- and long-term adverse effects of administering neuroprotective doses of stem cells or stem cell factors. One of the main concerns of stem cell transplantation is the development of neoplasms. It is believed that the tumorigenicity of UCB cells is less than that of embryonic or induced pluripotent cells (93), and a meta-analysis of over 1,000 subjects showed that intravenous or intra-arterial administration showed no association with the development of malignancy (94). Stem cell molecular factors alone may also pose risk, as BDNF and long-term IGF-1 therapy may be epileptogenic (95,96).

CONCLUSION

While stem cell therapy for neonatal and pediatric brain injury has demonstrated significant promise, many details of the therapy remain under investigation. For instance, to select ideal UCB units for therapy, numerous perinatal factors should be considered, most significantly the birth weight and gestational age at the time of birth. Future research should focus on the effects of perinatal factors on UCB stem cell function, as opposed to simply cell type and number, and attempt to better define the effects of race and ethnicity by obtaining more detailed demographic data or using genetic analyses. Early results from studies using stem cell–secreted neurotrophic and growth factors have shown similar success in attenuating brain injury; however, the short- and long-term adverse effects of these therapies have not yet been well evaluated.

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