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THE POTENTIAL FOR CELL-BASED THERAPY IN PERINATAL BRAIN INJURIES

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Abstract

Perinatal brain injuries are a leading cause of cerebral palsy worldwide. The potential of stem cell therapy to prevent or reduce these impairments has been widely discussed within the medical and scientific communities and an increasing amount of research is being conducted in this field. Animal studies support the idea that a number of stem cells types, including cord blood and mesenchymal stem cells have a neuroprotective effect in neonatal hypoxia-ischemia. Both these cell types are readily available in a clinical setting. The mechanisms of action appear to be diverse, including immunomodulation, activation of endogenous stem cells, release of growth factors, and anti-apoptotic effects. Here, we review the different types of stem cells and progenitor cells that are potential candidates for therapeutic strategies in perinatal brain injuries, and summarize recent preclinical and clinical studies.

Keywords

Stem Cell; Cerebral Palsy; neonate; brain injury; hypoxia ischemia

INTRODUCTION

Perinatally acquired brain injuries from stroke and hypoxia-ischemia are a leading cause of perinatal mortality and long term neurobehavioral morbidity [1] and a major public health issue worldwide. Neonates surviving the initial insult commonly experience severe chronic disabilities including cerebral palsy (CP), intellectual disability, seizure disorders and behavioral disorders. [2, 3]. Cell-based therapies have been widely discussed as potential interventions for perinatal brain injuries, however, only a few pilot trials have thus far been conducted. The objective of this article is to review the varieties of stem cells that have been studied as therapeutic options for CP and the preclinical and clinical evidence for their efficacy or limitations.

Cerebral Palsy as a Target for Cell Based Therapy

Cerebral palsy (CP) has been defined as a group of permanent disorders of movement and posture causing activity limitation that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain [4]. These non-progressive disturbances are moderate to severe perinatal insults in term and premature infants. In the term infant the

most common causes of the moderate to severe neonatal encephalopathy that can lead to CP are birth asphyxia, fetal inflammation and neonatal stroke [5–8]. In the prematurely born infant population, the more frequent forms of injury are to cerebral white matter, referred to as perinatal white matter injury or periventricular leukomalacia (PVL), as well as germinal matrix hemorrhage often leading to post hemorrhagic hydrocephalus. Other less common forms of perinatal brain injuries are infectious meningitis/encephalitis, hyperbilirubinemia-induced kernicterus, primary intraparenchymal hemorrhage, and cerebral sinus vein thrombosis. It is worthwhile to briefly define the pathology and clinical manifestations of the various conditions that can result in CP before considering cell-based therapeutic approaches.

Neonatal encephalopathy in the term infant that can present with an array of clinical abnormalities ranging from jitteriness to severe coma and seizures, is often a result of a hypoxici-schemic event that leads to focal necrosis due to energy failure and triggers an excito-oxidative cascade that propagates over several days leading to activation of multiple apoptotic pathways accompanied by an inflammatory response [2]. Neuroimaging and autopsy studies of term infants with moderate to severe encephalopathy from near total asphyxia, hypoxic ischemic encephalopathy (HIE) secondary to umbilical cord occlusion, often demonstrate relatively selective vulnerability of deep gray matter structures including the putamen and ventrolateral thalamus and the peri-rolandic cortex, and surviving infants frequently develop extrapyramidal cerebral palsy (ECP) [2, 5, 9–13]. ECP is manifested clinically by truncal hypotonia and dystonia combined with athetosis especially in the upper extremities, impaired speech, but relatively preserved intellectual ability. In contrast, babies that have been exposed partial-prolonged asphyxia, where there is less severe asphyxia extended over a period of several hours, develop injuries throughout the cerebral cortex but the basal ganglia are spared and they develop spastic quadriplegia and impaired intelligence [2, 14]. Neonatal ischemic stroke in term infants often presents with neonatal seizures, and is usually due to an arterial clot, resulting in wedge shaped focal infarction with loss of all cell types; as many as 25% of the surviving infants may develop hemiplegic cerebral palsy. Based on MR imaging approximately 80% of hemiplegic CP is not associated with neonatal signs, suggesting that the injury occurred prenatally.

In the preterm population, the targeted area is typically the immature periventricular white matter and can result in spastic diplegic CP. Extremely premature infants of 23 to 32 weeks gestational age are particularly susceptible to white matter injury due to the predominance of immature and differentiating oligodendrocytes in the cerebral white matter at that time [3, 15–18]. Prenatal exposure to placental or maternal inflammatory factors has also been implicated in increased risk of developing periventricular leukomalacia [19]. PVL lesions show loss of oligodendrocyte progenitor cells (OPC) and pre-Oligodendrocytes (pre-OL), surrounding the ventricles but also aberrant differentiation of these cells [3]. The autopsy of a prematurely born child with CP typically shows dysmyelination, reactive gliosis, white matter atrophy and ventriculomegaly [18, 20]. More recent studies have revealed the white matter injury to be paralleled by cerebral cortex and deep gray matter degeneration leading to neurobehavioral abnormalities [18, 20]. Germinal matrix hemorrhage is also associated with white matter injury with pathology similar to PVL [17, 21]. Given the ability of modern neuroimaging to diagnose these brain injuries in the neonatal period and later in life, often before signs of CP emerge, it would be attractive to develop cell-based therapies to reduce acute injury and/or repair brain injury after it has taken place but before long term changes in brain structure and function have emerged.

Defining stem cells and other multipotential cells used for therapy

Stem cells are classified as cells with self-renewing ability that can generate cells of more mature lineage. Stem cells can be generally classified into three groups, embryonic (ESC), somatic (SSC/ ASC) and reprogrammed (iPSC) and are summarized in table 1.

Embryonic Stem Cells (ESCs)—These cells are derived from fertilized germ cells prior to uterine implantation. ESCs derived from the zygote to the pre-implanted 3–4 day 4-cell embryo are considered totipotent or capable of developing into an embryo as well as the extra-embryonic placental tissue [22]. However, ESCs derived from the later stage inner cell mass of the 5–6 day old blastocyst, can give rise to the three germ layers, ectoderm, mesoderm and endoderm but are incapable of forming a placenta and are thus considered pluripotent (figure 1) [22]. First established from mice in 1981 and humans later in 1998, ESCs have advanced our knowledge of cell fate determination and maturation [23–26]. Human derived ESCs, which are derived primarily from in vitro fertilized embryos, have provoked an ongoing debate. Despite the promising early results, ESCs have not fulfilled their therapeutic potential for several reasons. The processes of manipulation to desired mature cell types are still not fully understood, and the allogeneic nature of ESCs creates a concern for immunologic reactions against them. In addition, their pluripotency is associated with high tumorigenicity, often leading to teratoma formation.

Induced pluripotent stem cells (iPSC)—These are mature, differentiated cells that have been induced to de-differentiate into ESC like cells in response to genetic manipulation. The most common method for creating iPSCs is by insertion of select genes such as Oct3/4, Sox2, Klf4, and c-Myc. into somatic cells. Insertion of these genes has been shown to induce pluripotency in embryonic and adult mouse fibroblast cells [27]. Follow up studies in adult human fibroblast cells confirmed the same four genes were sufficient to induce pluripotency [27, 28]. Induced pluripotent stem cells are important because they have been shown to develop characteristics seen only in germ cells such as formation of mesoderm, ectoderm and endoderm as well as teratomas. Induced pluripotent stem cells share many of the drawbacks of ESCs, such as tumor formation and a potentially high rate of chromosomal aberrations with each passage. However, the non-fetal and potentially autologous (self-derived) sources of these cells obviate the ethical challenge facing ESC use. The therapeutic potential is great for both ESC and iPSC cells but both require extensive further study before they can reliably and safely be utilized in clinical studies.

Somatic stem cells (SSC)—These cells are of non-germline origin and are found in most tissue in regions referred to as stem cell niches. SSCs, also referred to as adult stem cells (ASC), can be derived from aborted fetuses, umbilical cord blood, skin or bone marrow of individuals and even the brains of cadavers [29]. These cells are considered lineage restricted to the tissue type from which they were derived. Brain derived SSCs for example, are considered restricted to neural and endothelial cells and referred to as neural stem cells (NSC). These cells can be maintained and expanded in vitro until induced into a more mature phenotype with the appropriate bioactive molecules [30, 31]. However, in vitro studies have revealed SSCs may also be manipulated to alternate tissue types such as muscle, liver, blood, skin and endothelial cells [29, 32, 33]. These observations have been made in SSCs co-cultured with other cell types, for instance, NSCs cultured with cardiac or skeletal muscle gain myocyte characteristics [34, 35]. The actual mechanism through which this NSC plasticity is achieved has been proposed to be NSC acquisition of genetic lineage determinants by cell and/ or nuclear fusion [36–38]. Other studies have questioned this cell fusion hypothesis and instead proposed that expression of cell surface molecules and extracellular scaffolding are the determining factors behind the unexpected plasticity of some SSCs [33, 39].

One benefit of SSCs is they can be derived from their niches, potentially manipulated and transplanted back to the donor. Such an *ex vivo* application of autologous cells could significantly reduce the risk of rejection by the host. Further enhancing their promise, these SSCs do not exhibit the unlimited proliferative and tumorigenic properties of ESCs/ iPSCs. Since these cells can be derived from either renewable or non-viable sources, they are potentially powerful therapeutic tools [40]. NSCs have been derived from human cadaver tissue and initial human pilot transplant studies with direct intracerebral delivery of these cells are ongoing in the United States although only for Parkinson's disease, not for cerebral palsy or perinatal brain injury studies [41].

The most commonly used and probably best described of the SSCs are hematopoietic (HSC) and mesenchymal (MSC) stem cells. HSCs are found in bone marrow niches and in umbilical cord blood (UCB) and can also be derived from peripheral blood after bone marrow stimulation with granulocyte colony stimulating factor (G-CSF) [42, 43]. While HSCs can be defined by expression of a single glycoprotein CD34, MSCs have a more complex pattern of marker expression. HSCs are capable of differentiating into the various myeloid and lymphoid lineages while MSCs mature to the various mesodermal lineages including adipocytes, fibroblasts and osteoblasts. MSCs are derived from the bone marrow, skin adipose tissue, umbilical cord blood, and in highest concentration from Wharton's jelly. A recent study showed that a fraction of perivascular cells in the brain are MSCs [44, 45]. There is ongoing debate on whether MSCs and HSCs can trans-differentiate into non-hematopoietic or non-mesenchymal lineages such as neural [24].

Another type of SSC that warrants discussion here is the glial restricted precursor (GRP). These cells can be derived from the CNS of E12–13 rodent fetuses and from aborted human 14–18 week old fetuses. GRPs are early precursors within the oligodendrocytic and astrocytic cell lineages and are defined by their expression of A2B5 [46, 47]. In the appropriate medium, these cells begin expressing the early oligodendrocytic lineage markers PDGFR and NG2 and have recently been shown to become mature oligodendrocytes *in vivo* [48]. These precursor cells are currently being studied as a potential source for cell-based therapeutic approaches in disorders of the central nervous system white matter, including multiple sclerosis, and leukodystrophies, and are of particular interest for periventricular leukomalacia of the preterm infant [49].

Finally, another type of SSC, referred to as Olfactory Nerve-Ensheathing cells (OEC), reside in the olfactory epithelium and continue to proliferate throughout life. OECs exhibit both astrocyte and Schwann cell properties and have been observed to migrate to the olfactory bulb [50]. OECs have exhibited Schwann cell like myelination and promote axonal regeneration and conduction in demyelinated and transected CNS axons, [51] reviewed in [52]. In addition to their ability to integrate into host tissue, OECs also express trophic factors including NGF, BDNF and GDNF along with their cognate receptors [53]. Although OECs don't necessarily fit the classic description of stem cells, their characteristics make them feasible candidates for cell therapy as well as research into the mechanisms controlling myelination.

STEM CELL THERAPEUTIC STRATEGIES

Autologous Versus Allogeneic Cells—Cell transplantation can utilize either autologous (cells are returned to the donor's body) or allogeneic (cells are taken from a human donor and reinfused to a human recipient) donor sources. Xenotransplant refers to transplantation of cells derived from a different donor species and is not utilized in human diseases. In the case of allogeneic transplantation, one of the major complications is the immune mediated response to the foreign tissue. The immune responses may occur as a host

vs. graft response, defined as the mediation of an inflammatory response against the donor cells in the recipient; this will lead to rejection of the donor stem cells. The second possible immune response is called graft vs. host disease (GVHD), in which donor derived T-cells attack the recipient's organs. GVHD can present acutely with a severe rash, high fever, diarrhea and damage to the liver. If GVHD lasts over 100 days, it is defined as chronic GVHD, and affects not only the above mentioned organs but also connective tissues and exocrine glands. In order to avoid these severe complications, extensive human leukocyte antigen (HLA) serotyping is performed. While there are over 2500 HLA molecules, only a handful are considered major, and the more HLA molecules two people share the less the chance for GVHD. Interestingly, cord blood transplantation has a lower rate of GVHD compared to bone marrow transplantation and therefore requires a less stringent HLA match. In addition to vigorous HLA typing, allogeneic transplantation also requires a high level of host immunosuppression making the subjects prone to opportunistic infections and chemotherapy associated complications.

Transplantation in Relationship to the Evolution of Injury—The approach to cell-based therapy for perinatal brain injuries is dictated by the pathophysiology and course of the target disease. The strategy can be either neuroprotective or restorative in nature depending on the time of intervention. In term infants, with hypoxic ischemic encephalopathy (HIE) or neonatal ischemic stroke, where there is an acute timed insult determined by the baby's presentation and neuroimaging studies, exogenous cells could exert a neuroprotective effect when given during the acute phase. The protective effect would be mediated by providing trophic support and/or amelioration of the inflammatory response following a hypoxic-ischemic event. The exogenous cells could also potentially lead to repair by stimulating the endogenous stem cell niches to proliferate and replenish the populations of pre-OLs and neurons which are depleted by hypoxia-ischemia induced events in the term infant. The tissue microenvironment intrinsic to the disease process, the type of cell used and the route and the timing of intervention are major factors to be considered in any cell-based therapy trial. Therefore, it is crucial that appropriate animal models are developed, and that these factors are delineated first in these preclinical models prior to moving to human trials. However, while many animal models for various forms of perinatal brain injuries exist, most studies are limited to short term outcome. Many of the published injury models either exert very limited long-term changes, or are too severely injured to be maintained for a long-term study.

In the preterm population, in contrast, there is often no clear acute encephalopathy, and many babies do not present with symptoms of cerebral palsy until much later in infancy or early childhood years. During these chronic stages, there is extensive atrophy and gliosis of the white matter and one would have to consider exogenous stem cells with restorative potential, such as cells that would supplement the injured white matter. Furthermore, the injured brain microenvironment, which has excessive gliosis in the chronic stages, may have an altered local pattern of growth factors and intercellular matrix proteins that may not be suitable for exogenous cells to proliferate or differentiate into the desired cell type. Given these factors, the timing of transplantation is a crucial factor. A study of NSC transplantation in a neonatal ischemic stroke mouse model, showed that intrastriatal injections of these cells into pups with unilateral carotid artery ligation reduced the degree of brain atrophy, when performed 2 days after injury but not when performed 7 days after injury [54]. This result is noteworthy because the therapeutic window provided by NSCs, from initial hypoxia-ischemia to treatment, is considerably longer than that provided by neuroprotective drugs. In contrast, a study in spinal cord T10 injury model injury in rats showed that human NSCs survived and differentiated into neurons and oligodendrocytes when transplanted into the moderately contused spinal cord at 9 days post injury, while grafting hNSCs on the day of or 3 days after injury resulted in no or very few surviving cells [55]. It is likely that time

dependent changes in the microenvironment such as degree of excitotoxicity, oxidative stress, and growth factor release, determine the efficacy of transplanted cells.

Preclinical Studies of Cell Based Therapies for Perinatal Brain Injuries

Embryonic Cortical Graft Transplantation—One of the earliest studies into the feasibility of cell based therapy of hypoxia-ischemia (HI) utilized transplantation of neocortical grafts from E13 rats into 2 week old rat models of unilateral (HI) [56]. Cortical grafts removed from E13 rat embryos consisted of a mixed population of neural stem cells including glial restricted precursors and pre-oligodendroglia. These grafts contained cells derived from the neocortex of rat embryos that were mechanically dissociated, then maintained in culture before being stereotactically injected into the hypoxic-ischemic parenchyma [56, 57]. The techniques used in this study were widely adopted from earlier transplantation studies of rodent models of other diseases such as Parkinson's [58–61]. Animals were assessed histologically 2–6 weeks following transplantation and graft survival was found in 80% of animals [56]. Further, there was evidence of graft integration with the host tissue by the presence of acetylcholine-esterase-positive fibers [56, 61]. This survival and integration did not reduce HI mediated cell loss in select brain regions in the time period assessed although longer survival times may be more revealing. Since only histological and morphometric assessments were performed it is not clear whether behavioral improvements were achieved in this study. One subsequent study transplanted neocortical sensorimotor suspensions, derived from E16 rat fetuses, into a 10 day old unilateral HI rat model. Observers reported improved sensorimotor and locomotor function as determined by Rota-Rod and apomorphine stimulated rotations tests [62]. These early studies helped to establish the feasibility of transplantation studies by showing that transplanted grafts can survive and integrate into host tissue. The excitement generated by these earlier studies has catalyzed further exploration of the therapeutic potential of stem/ progenitor cells in perinatal brain injuries.

GRP Cell Transplantation—White matter injury of the preterm infant may have many similarities with other white matter diseases such as multiple sclerosis and leukodystrophies where cell-based remyelination strategies have been pursued in preclinical studies [49]. The shiverer mouse is the mouse model for the dysmyelination of many of the leukodystrophies. The animal is deficient in myelin basic protein (MBP), as a result of a premature stop codon in the MBP gene [63, 64], and affected mice typically die within the first few weeks of life. Studies in this model have highlighted the potential of using cell replacement therapy to remyelinate lesioned sites. First demonstrated by Lachapelle in 1983, the myelination potential of grafting GRPs into shiverer mice has since been repeatedly demonstrated [65]. Studies with SubVentricular Zone progenitors have also produced similar results in a myelin deficient rat model [66].

A more recent study using human oligodendrocyte progenitors (hOPCs) from adult or 21–23 week fetuses showed higher levels of migration and integration of these cells into the shiverer mouse brain compared to earlier studies done at 4 weeks post-transplant. Human OPCs were observed myelinating shiverer axons starting at about 8 weeks and maximizing at 12 weeks post-transplant [67, 68]. In addition, a significant number of hOPC transplanted shiverer animals, demonstrated significantly increased lifespans [67]. Later studies with human glial restricted precursor cell (hGRP) transplantation into shiverer mice showed great level of integration of these cells into the white matter with extensive myelination of the CNS. However, the same cells when transplanted into rats with demyelinated spinal cords did not differentiate into oligodendrocytes but appeared to become astrocytes instead. Interestingly, despite the lack of remyelination in these rats, the hGRP treated group had improved electrophysiological function [48].

Another important study reported mixed glial culture derived cell transplantation in P11 rat pups that were injected intracranially at P5 with lipopolysaccharide (LPS). This LPS induced inflammation generated at sub-chronic rat model of PVL [18]. Interestingly, the exogenous cells which were derived from GFP expressing rat neonates, were able to survive for at least 8 weeks and had committed to glial progeny, including astrocytes and mature oligodendrocytes. Furthermore, OPC transplanted animals had less LPS-induced neuronal loss and higher counts of endogenous oligodendrocytes compared to untreated injured animals. While this study is highly relevant to PVL, one weakness of this study is that the intracerebral cell transplantation was performed during the early neonatal period, a scenario that is unlikely to be feasible in the clinical setting, since the premature newborn would often be too sick to tolerate such an invasive procedure, and the current clinical and neuroimaging tools are imperfect predictors of outcome at this early age.

One of the notable observations on stem cell use in experimental models is the consistent migration from the graft site to the lesioned area when the two are spatially separated, for example on opposite sides of the brain. This phenomenon has been demonstrated in a number of studies. Transplantation of multipotential astrocytic stem cells into a rat HIE model demonstrated this migration and a percentage of the cells developed neuronal phenotypes [69]. This targeted migration of stem cells may allow their use as a platform for drug delivery which obviates the side effect inducing nature of current non-specific drug delivery mechanisms [70, 71]. While intracerebral transplantation is currently the most efficient method to deliver large quantities of cells to the lesioned brain, other methods are being used that take advantage of the cells' migratory qualities. Intravenous, intraperitoneal and intrathecal delivery of exogenous cells are other less invasive approaches for cell delivery and circumvent the invasive nature of intracerebral transplantation protocols. However, with intravenous or intraperitoneal delivery, many cells are found in the body's filters such as the spleen and lungs and often few or no cells are found in the brain [72, 73].

Transplantation of HUCBCs and MSCs in Preclinical Models—The usefulness of stem cells derived from bone marrow and umbilical cord blood (HUCBC) are the focus of intense investigation. These HSCs and MSCs are being studied for their therapeutic potential in trials for conditions ranging from MS and ALS to cerebrovascular disease [32, 74, 75]. First reported in 2006, mononuclear cells derived from HUCBCs were delivered IP in rat models of unilateral HI injury and alleviated spastic paresis improving motor performance [76]. While HUCBC derived cells were found in the lesion, none expressed neural markers and the studies ended only 2 weeks after cells were delivered. Other studies have reported similar observations of an apparent homing ability of these stem cells. In general, cells seem to migrate to the CNS despite being injected in peripheral locations [74, 77, 78]. Another key observation from these studies is the apparent suppression of inflammation in the study subjects. Transplanted MSC and HSC derived from marrow and cord blood had anti-inflammatory and anti-apoptotic effects on the host tissue [79–81] despite their allogeneic and even xenogeneic properties. Another study utilized intracerebral transplantation of MSCs, derived from the bone marrow of GFP expressing mice, into the ipsilateral hemisphere of a unilateral rat HI model [82]. Transplanted 3 and 10 days post HI to explore the limits of the therapeutic intervention window, animals showed reduced forepaw asymmetry in rearing tests, increased proliferation and differentiation in cortex and reduced lesion size. Notably, animals receiving MSCs 3 days post HI exhibited more robust effects than the 10 day post HI group (figure 3) and the ratio of proliferating cells that were also GFP positive was very low and not included in the assessments [82].

Although the mechanism by which these cells provide protection is not fully understood, studies are indicating that the heterogeneous nature of the pool of cells used provide a wide range of trophic factors and other bioactive molecules which are the actual driving force

behind the improved outcomes seen in animal studies [83–86]. This phenomenon has also been used to describe similar observations with other classes of stem cells including NSCs. The delivery of trophic factors by these cells is significant since there is often reported neurobehavioral recovery and reduced inflammation even when there is minimal migration of injected cells to the injury site [72]. While these investigations have provided promising results, it remains to be determined whether the effects are only transient or can achieve complete functional recovery.

The migratory propensity of transplanted cells can be combined with their multipotential nature to create customized cells for clinical interventions. This synergism of seemingly unrelated characteristics could provide powerful targeted delivery mechanisms and possibly reduce unwanted side effects. Transplanted NSCs tend to migrate apparently because they express CXCR4 which is the cognate receptor for the α -chemokine, stromal-derived factor (SDF-1 α). In the presence of SDF-1 α , a CXCR4 mediated cascade activates migration, proliferation and engagement pathways in quiescent NSCs [87, 88]. Neural stem cells could be primed prior to transplantation with CXCR4 agonists to optimize the migratory potential of the NSCs. Furthermore, the multipotential nature of MSCs and NSCs can be harnessed by engineering expression of select transgenes to customize differentiation to match the clinical need. It has been reported in mouse models that NSCs express the neurotrophin 3 (NT-3) receptor, TrkC. Experiments using an NSC line, engineered to express NT-3, survived, migrated and preferentially differentiated into cholinergic, glutamatergic and GABAergic neuronal subtypes as determined by their respective expression of vesicular acetylcholine transporter, glutamate and GABA [71]. Based on these data, NSCs and stem cells in general could be engineered to express genes that will maximize differentiation to sub-populations lost to injury.

Clinical trials for perinatal brain injuries and CP

Clinical stem cell transplantation, with bone marrow derived cells, has been performed for decades, and is currently standard of care for a number of hematologic and oncologic conditions and also increasingly for some neuro-metabolic diseases. The growing body of data also being generated by pre-clinical studies is serving as the basis for a small number of limited clinical studies. There are currently six trials listed on clinicaltrials.gov in which stem cells, derived from HUCBC or bone marrow, are being assessed for safety and therapeutic efficacy in HI injury and CP (table 2). Studies are also ongoing in leukodystrophies such as Pelizaeus-Merzbacher disease where human NSCs were transplanted intracranially into the frontal lobe white matter of patients [75]. Recipients were 6 months to 5 years old and were immunosuppressed for 9 months and subject to neurological and MRI evaluations. All four subjects showed motor and mental status improvement, 3 showed modestly improved neurological function and 2 showed improved truncal support [75]. The observations made in leukodystrophy studies will likely be adapted to studies of HI mediated injury.

Human Umbilical Cord Blood Cell Transplantation in Clinical Trials—One recently completed trial in South Korea assessed the combined effectiveness of allogeneic umbilical cord blood stem cells in parallel with erythropoietin (EPO), which has a neuroprotective effect, in children 10 months to 10 years of age with CP. Patients received a one-time IV delivery of 3×10^7 /kg cells along with EPO delivered IV then subcutaneously for 1 month. Immune response to the allogeneic graft was suppressed with 1 month IV delivery of cyclosporine. So far these data have not been published.

Bone Marrow Derived HSCs—Another recently completed clinical study of CP include intrathecal delivery of CD34⁺ and CD133⁺ bone marrow derived hematopoietic stem cells to

respectively assess developmental delays and safety and side effects. An ongoing clinical trial is examining whether reinfusion of autologous umbilical cord blood can effect improvement in neurodevelopmental function in full term newborns with neonatal encephalopathy. Two other ongoing clinical trials are studying the safety and efficacy of autologous cord blood hematopoietic stem cells in CP. Patients are generally assessed with a number of tools including measures for gross motor performance and gross motor function. Other assessment tools include the Amiel-Tison Neurological Assessment and the Battelle Developmental Inventory. Although little data have been reported in these juvenile CP trials the strong likelihood of a graft vs. host response in allogeneic transplants remains a great concern, and requires extended use of immunosuppressing agents that alone cause side effects but also may exacerbate the neurologic deficits.

Olfactory Epithelial Cells—A recent study reported the effects of transplanted human olfactory epithelial cells OECs in patients with CP, evaluated in regards to safety and therapeutic effectiveness [89, 90]. Six patients aged 1 to 12 received allogeneic OECs (derived from aborted fetuses and HLA matched to recipients) stereotactically implanted into the corona radiata of the frontal lobe [89]. (OECs) comprise a small part of the spectrum of candidate cells being used in cell replacement therapy studies but are generating excitement based on their lineage and growth factor secretion characteristics eight patients were used as historical controls, however, their level of motor disability was significantly less than the transplanted group. The authors stated that no immunosuppression was performed. The authors further stated that patients who received OECs exhibited no side effects and showed a mild but significant improvement in motor function scores after 6 months post OEC transplantation [89]. However, due to the very small sample size, one has to be cautious in the interpretation of the results.

SUMMARY

Pre-clinical studies show that cell-based therapies have the ability to protect and/or repair brain tissue in neonates following hypoxic-ischemic brain injuries. Stem cells have the ability to migrate directly to the injured brain when administered intracerebrally or systemically. The diversity of targets for stem cell therapy underscores the promise of this rapidly growing field. As the mechanisms are better understood and better animal models developed we can expect greater pressure for more clinical trials that take advantage of this therapeutic tool for CP and related disorders. Also, the lack of effective interventions for many other neurodegenerative diseases and the potential of cell-based personalized treatments are creating excitement in the field. Safe and effective clinical interventions with cell-based therapies remain for the future, and it important for cell-based therapies to be tested in well designed controlled trials, before they are disseminated as useful therapies in humans. However, this area is one that carries hope.

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Figure 1. Various types of Stem Cells

Adapted from: Mitalipov S, Wolf D. Adv Biochem Eng Biotechnol. 2009;114:185–99.

Figure 2. Fate of transplanted oligodendrocyte progenitors in a rat model of periventricular leukomalacia (adapted from Webber et al. *Am J Pathol.* 2009 December; 175(6): 2332–2342) GFP-OPCs survive transplantation in a LPS induced rat PVL model for at least 8 weeks and commit to glial progeny. GFP cells were clearly in both gray and white matter at 8 weeks post-transplantation in lesioned animals (arrows) and were identified to express NG2 (arrowhead, red, B), a marker for OPCs, Olig2 (arrowhead, red, C), a marker for oligodendrocytes, and GFAP (arrowhead, red, D), a marker for astrocytes. Co-staining for the neuron specific marker, NeuN, was not observed at 8 weeks (E). The authors reported that the majority of GFP-positive cells co-labeled with Olig2 and NG2, while none was NeuN co-labeled (F).

Figure 3. Effect of intracerebral MSC treatment at 3 days after unilateral HI on lesion size and functional outcome (adapted from van Velthoven et al., *Brain, Behavior, and Immunity* Volume 24, Issue 3, March 2010, Pages 387–393)

MSC treatment reduced HI- induced Microtubule Associated Protein 2 (MAP2)+ area loss (A) and Myelin Basic Protein (MBP)+ area loss (B) expressed as ratio ipsi-/contralateral area. Furthermore, paw preference in the cylinder rearing test was determined as a measure of lateralizing motor deficits at 10 and 21 days after the insult and was significantly improved in cell treated animals (C). Data represent mean percentage area loss $\pm$ SEM or mean percentage difference between non-impaired and impaired forepaw initiation $\pm$ SEM. N = 10–12 animals per group. (● $p < 0.05$; ●● $p < 0.01$).

Table 1

Characteristics of Stem Cells and Sources.

	ESC	SSC/ASC	iPSC
Tumorigenicity	High	No risk	High
Ethical Constraints	High	None	Low/ none
Source	Embryo/ Blastocyst	Stem cell niches in brain/ bone marrow/ Cord blood etc.	Fibroblasts or any other cell

Table 2

Typical applications of bone marrow and cord blood-derived cells in therapy of neonatal hypoxia-ischemia models.

Experimental Design	Cell Type (Marker)	Delivery Route	HI Model	Total Cell Dose	Does Interval	Summary
De-differentiated/reprogrammed MSCs overexpressing bcl-2 and MiR-34a [91]	Rat BM MSC: CD29 ⁺ , CD90 ⁺ , CD106 ⁺ , CD34 ⁺ , HLA—DR ⁺ , CD45 ⁺	Transplanted into right lateral cerebral ventricle;	Right CCA ligation of P7 SD rats 2hr recovery and 2hr hypoxia (8% O ₂ in N ₂)	1-2 × 10 ⁵ MSCs in 5 µL PBS	5d post HI	Cells differentiated into neurons and endothelial cells 7d post injection, Memory function improved assessed 2 month post-tx
Intranasal MSC delivery to hypoxic ischemic mice [92].	Mouse BM-MSC: Sca-1 ⁺ , CD90 ⁺ , CD29 ⁺ , CD44 ⁺ , MHC-I ⁺ ; No hematopoietic or Myeloid cells	Intranasal delivery	Right CCA occlusion of P9, C57B1/6 mice and 45 min hypoxia (10% O ₂ in N ₂)	2.5 × 10 ⁵ MSCs in 6µL PBS	10d post HI	Cells improved cylinder rearing test findings 3 weeks post-tx, and reduced brain lesion size 3-4 weeks post-tx
Transplanted MSCs as a standard experimental method [82].	Same as above	Transplanted into ipsilateral hemisphere	Same as above	100,000 MSCs in 2µl PBS injected over 4min.	3d post HI and 10d post HI	Cells reduced lesion size even when given 10d post HI, increased neurogenesis, decreased microgliosis
IntraCardiac (IC) delivery of human MSCs (hMSC) in a rat HI model [93].	Human BM-MSC: CD73 ⁺ , CD105 ⁺ , CD14 ⁺ , CD34 ⁺ , CD45 ⁺	IC injection	Severed Left CCA of P7 SD rats, 2hr recovery and 3.5hr hypoxia (8% O ₂)	10 ⁶ MSCs injected over 1min	72hr post HI	Cells reduced lesion size, improved cylinder test performance
hUCB delivered IP in rat HI model [72].	hUCB mononuclear cells	IP injection	Right CCA ligation of P7 male LH rats, 2hr recovery and 90min hypoxia (8% O ₂ in N ₂)	2 × 10 ⁶ cells in 200µL DMEM	3hr post HI	Improved developmental sensorimotor reflexes and reduced striatal necrosis 1 week after injury, also reduced microglial activation
IP transplanted hUCB cells in a rat HI model [76].	hUCB and placenta derived mononuclear cells	IP injection	Severed Left CCA P7 rats, 1hr recovery and 80min hypoxia (8% O ₂ in N ₂)	1 × 10 ⁷ hUCB-derived mononuclear cells in 500µL 0.9% NaCl	24hr post HI	Reduced spastic paresis assessed 2 weeks post-tx, no change in pathologic injury severity, cells entered CNS

abbreviations: CCA-common carotid artery; BM-bone marrow; hUCB-umbilical cord blood; IN- intranasal; IC- intracardiac; SD Sprague Dawley