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Stem Cell Therapy for Neonatal Disorders: Prospects and Challenges

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Despite recent advances in neonatal medicine, neonatal disorders, such as bronchopulmonary dysplasia and intraventricular hemorrhage in preterm neonates and hypoxic ischemic encephalopathy in term neonates, remain major causes of mortality and morbidities. Promising preclinical research results suggest that stem cell therapies represent the next breakthrough in the treatment of currently intractable and devastating neonatal disorders with complex multifactorial etiologies. This review focuses primarily on the potential role of stem cell therapy in the above mentioned neonatal disorders, highlighting the results of human clinical trials and the challenges that remain to be addressed for their safe and successful translation into clinical care of newborn infants.

Key Words: Mesenchymal stem cells, bronchopulmonary dysplasia, intraventricular hemorrhage, hypoxic ischemic encephalopathy, newborn

INTRODUCTION

Despite recent advances in neonatal intensive care medicine, several neonatal disorders, including bronchopulmonary dysplasia (BPD)[1,2] and severe intraventricular hemorrhage (IVH)[3] in preterm neonates and hypoxic ischemic encephalopathy (HIE)[4] in borderline preterm and term neonates, remain major causes of mortality and morbidities. Few effective therapies are currently available to ameliorate the injuries resulting from these disorders. Therefore, developing new safe and effective therapies to improve the outcomes of these intractable and devastating neonatal disorders is an urgent and considerable issue.

Recently, various preclinical studies have shown that stem cell therapy significantly attenuates injuries in newborn animal models of BPD,[5] HIE,[6] and IVH.[7] Phase I clinical trials conducted in neonates with BPD,[8] HIE,[9] or severe IVH (NCT02274428) have shown stem cell treatments to be safe, feasible, and potentially efficacious. Overall, these findings suggest that stem cell therapy might represent the next breakthrough in therapy of these currently intractable and devastating neonatal disorders with complex multifactorial etiologies. This review summarizes recent advances in stem cell research for treating neonatal disorders of BPD, HIE, and IVH. We focus on the results of human clinical trials and the challenges, including safety and delivery of the optimal cells for the right patient with the right route at the right time and dose, that remain for the safe and successful translation of stem cell therapies into clinical care of newborn infants.

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CLINICAL TRIALS DATA

Phase I clinical trial for BPD

Based on preclinical evidence showing the protective effects of mesenchymal stem cell (MSC) transplantation against neonatal hyperoxic lung injuries without short- and long-term toxicity or tumorigenicity,[5,10-13] the first pioneering clinical study in preterm infants at risk of BPD has been conducted.[8] In an open-label, single-center, phase I, dose-escalation clinical trial, Chang, et al.[5] assessed the safety and feasibility of a single intratracheal (IT) transplantation of allogenic human umbilical cord blood (UCB)-derived MSCs for BPD. The MSCs were transplanted into nine very preterm infants at high risk for developing BPD,

with a mean gestational age of 25 weeks (range: 24–26 weeks) and a mean birth weight of 793 g (range: 630–1030 g) at a mean age of 10 days (range: 7–14 days) after birth. The preterm infants were all on continuous ventilator support with no signs of imminent clinical improvement. Three patients received a low dose (1×10^7 cells/kg in 2 mL/kg of saline), and the remaining 6 patients received a high dose (2×10^7 cells/kg in 4 mL/kg of saline). The MSCs were administered intratracheally in two fractions into the left and right lungs via a gavage tube using the same method for administering surfactant. The treatment was well tolerated without any serious adverse effects or dose-limiting toxicity up to 84 days following transplantation. No significant differences in serious adverse effects were observed between the low- and high-dose groups. Levels of cytokines in tracheal aspirates at day 7 following treatment were significantly reduced, compared with those at baseline. Furthermore, BPD severity was significantly lower in transplant recipients, compared with the historical gestational age-, birth weight-, and respiratory severity score-matched control group. Although it is too early to draw any firm conclusion on therapeutic efficacy, the study indicates that IT transplantation of allogenic human UCB-derived MSCs in very preterm infants at highest risk for developing BPD is safe and feasible. A long-term follow-up study and a phase II, double-blind, randomized, controlled trial to assess therapeutic efficacy are underway.

Phase I clinical trial for HIE

To date, no clinical trial using MSCs for HIE has been reported, and one phase I clinical trial using autologous UCB cells has been reported.[9] Cotten, et al.[9] assessed the safety and feasibility of autologous UCB cell transplantation in a phase I clinical trial of 23 neonates with HIE who met criteria and received concurrent hypothermia treatment. In that open-label study, intravenous infusion of non-cryopreserved, autologous, volume- and red blood cell-reduced UCB cells, up to four doses of 1–5×10^7 cells per dose, was found to be feasible and well tolerated. With appropriate isolation and processing, collection of UCB was successful even with a small volume of cord blood (range: 3–178 mL) to achieve the goal dose for each infusion for HIE in near-term and term infants with hypothermia. No significant adverse reactions, cardiopulmonary compromise, or infections associated with the transfusion were observed, and UCB recipients showed better 1-year survival rates (74%) with Bayley scores >85 than concurrent cooled infants (41%). Collectively, these findings suggest that collection, preparation, and intravenous infusion of fresh, non-cryopreserved, autologous UCB cells for clinical use in infants with HIE are likely safe and feasible. Randomized, double-blind, controlled clinical trials in the future will be required to evaluate the therapeutic efficacy of autologous UCB cell therapy for HIE.

Phase I clinical trial for IVH

Recently, a phase I, dose-escalating clinical study on the safety and feasibility of human UCB-derived MSC transplantation in preterm infants with severe IVH (i.e., grade≥3) (NCT02274428) has been conducted in light of promising preclinical evidence demonstrating the protective effects of MSC transplantation in a newborn rat model of severe IVH.[7] This study was an open-label, single-center clinical trial to assess the safety and feasibility of a single intraventricular transplantation of allogenic human UCB-derived MSCs within 7 days after detection of severe IVH (grade≥3) under ultrasound guidance in preterm infants. Of a total of nine preterm infants with severe IVH, three were given a low dose (5×10^6 cells/kg in 1 mL/kg of saline); after confirming that no dose-limiting toxicity or serous adverse events were associated with transplantation, the remaining six infants were given a high dose (1×10^7 cells/kg in 2 mL/kg of saline). The primary outcome measures were unsuspected death or anaphylactic shock within 6 h after MSC transplantation, and the secondary outcome measures were death or hydrocephalus requiring shunt surgery up to 1 year of age. These enrolled infants are currently undergoing follow-up care for long-term adverse outcomes and assessment of neurologic health. We are also planning to conduct a double-blind, randomized, phase IIa, controlled clinical trial to test the therapeutic efficacy of MSC transplantation for currently intractible and devastating severe IVH in premature infants.

PROSPECTS AND CHALLENGES FOR CLINICAL TRANSLATION

Clinical translation of stem cell therapies could represent a paradigm shift in neonatal medicine. However, barriers to the clinical implementation thereof, including delivering optimal cells for the right patient with the right route at the right time and dose and long-term safety of MSC transplantation, need to be resolved for successful clinical translation of stem cell therapies in the near future. Current research on each barrier is briefly summarized and discussed in the following sections.

The right cells

Choosing the right cells for transplantation is the most critical issue for successful clinical translation of stem cell therapy. As of today, MSCs have been most extensively investigated, since they are easily obtainable, do not have the ethical or safety concerns (e.g., tumorigenic potential) of embryonic stem cells, and their therapeutic efficacy has consistently been demonstrated in animal models of BPD,[5,10,12-15] HIE,[6] and IVH.[7,16,17] Although no specific markers for MSCs exist, as of now, MSCs have several unusual characteristics: 1) fibroblast-like morphology, 2) adherence to plastic, 3) positive expression of CD73, CD90, and CD105, 4) negative expression of CD45, CD34, CD14, CD11b, and HLA DR, and 5) differentiation capacity into various cell lineages including adipocytes, chondrocytes, and osteoblasts.[18]

Use of allogenic versus autologous MSCs is one issue to be addressed. MSCs are known to be immune-privileged due to their lack of MHC II antigens and their ability to inhibit the proliferation and function of immune cells, such as dendritic cells, NK cells, and T and B lymphocytes,[19,20] and these features allow for easier allogenic therapy.[21] In addition to their immune tolerance, donor MSCs do not engraft, and they fade away after therapeutic paracrine protection,[22] such that immunosuppression of the recipient might not be necessary. Therefore, allogenic transplantation of MSCs could safely be applied to all neonates in need of this therapy. Furthermore, considering the time-consuming and costly isolation and expansion processes for autologous MSCs, allogenic therapy might have a logistic advantage, because they are ready to use "off the shelf" in the clinical setting.

As MSCs are broadly distributed in the body, determining the tissue source of MSCs is another issue that needs to be resolved. Gestational tissues, such as the placenta, amniotic fluid, UCB, Wharton's jelly, and the umbilical cord, are medical waste and usually discarded at birth. Therefore, MSCs obtained from gestational tissues are particularly attractive due to their easy obtainability and lack of significant ethical concerns. Furthermore, MSCs derived from gestational tissues have been shown to exhibit lower immunogenicity,[23,24] higher proliferative capacity,[25,26] and higher paracrine potency than adult tissue-derived MSCs.[27] UCB-derived MSCs have been found to offer better *in vivo* therapeutic efficacy in attenuating hyperoxic lung injuries and increased paracrine potency, compared to fat-derived MSCs, in newborn rats.[15] Overall, these findings suggest that gestational tissues, rather than adult tissues, might be a promising source for obtaining exogenous human MSCs for future clinical use in treating neonates with intractable disorders.[14,15]

Production of a high-quality, standardized, clinical-grade product using good manufacturing practice (GMP) criteria is another critical issue for the success of MSCs in clinical settings.[28] Allogenic human UCB-derived MSCs manufactured in strict compliance with GMP criteria and approved by both Korean and US FDAs were used for phase I/II clinical trials for BPD conducted in Korea[8] and the USA (NCT02381366) and for a phase I clinical trial for severe IVH (NCT02274428). Important quality control criteria include the risk for malignant transformation, as well as aging, of the manufactured MSCs.[8] We have observed karyotypic stability and no senescence up to the 11th passage of human UCB-derived MSCs, and sixth-passage MSCs were used for the clinical trial to avoid these potential risks.[8] Standardization of isolation and expansion procedures, minimized batch variability, increased cell potency, and mass production of clinical-grade products in GMP-compliant facilities would be necessary for future clinical introduction of other cell types, including endothelial progenitor cells and amnion epithelial cells, as well as cell-derived products, such as exosomes, that show beneficial effects in models of neonatal disorders.

The right patients

Despite preclinical and clinical evidence demonstrating potential benefits, cautious risk-benefit evaluations must be made before implementing routine clinical use of MSC transplantation in vulnerable preterm infants. Therefore, identifying newborn infants at highest risk for developing intractable diseases and the ensuing mortality and morbidities is very important for applying these potentially beneficial rescue/preventive strategies.

As younger gestational age and prolonged respiratory support are two prime predictors of subsequent development of BPD in extremely preterm infants,[29] extremely preterm infants at 24–26 weeks gestation needing continuous ventilator support without clinical improvements within 24 hours were enrolled in the phase I clinical trial conducted by Chang, et al.[8] However, clinical courses can widely vary, and not all of these high-risk extremely preterm infants develop BPD. Additional clinical predictors and/or biomarkers[30] will be necessary to identify infants that will ultimately progress to BPD in order to avoid unnecessary treatment exposure.

Currently, although hypothermia is the only clinically available treatment for neonatal HIE, it is only partially effective, especially in severe cases.[31,32] Therefore, early identification using not only categorical neurologic examination, but also biomarkers of neuronal injury[33] in neonates with severe HIE who ultimately might benefit from stem cell therapy, in addition to hypothermia,[6] is crucial for better therapeutic outcomes.

More than 50% of preterm infants with severe (grade≥3) IVH die or develop posthemorrhagic hydrocephalus (PHH), which requires shunt surgery in up to 70% of cases.[34] Therefore, severe IVH in preterm infants might be a good therapeutic indication of stem cell therapy. Further studies will be necessary to clarify whether biomarkers of neuronal injury in cerebrospinal fluid[35] can predict the progress of PHH and poor neurodevelopmental outcomes, thus stratifying the preterm infants with severe IVH who might most benefit from stem cell therapy.

The right route

Various preclinical and clinical studies of BPD, HIE, and IVH have attempted to determine the optimal route for MSC translation using several routes, including local intraventricular,[7,36] intrathecal,[37] intranasal,[38] or IT[5] administration and systemic intraperitoneal[5] or intravenous[9,39] administration.

Although intranasal delivery of MSCs is minimally invasive, it did not attenuate hyperoxic lung injuries in mice[40] and showed less therapeutic efficacy requiring more than five-fold dose in attenuating brain injuries in HIE.[41,42] Therefore, more preclinical studies in larger animals, such as piglets or primates, verifying the therapeutic efficacy of intranasal delivery of stem cells are necessary for successful clinical translation.

As systemically transplanted MSCs migrate and localize toward injured tissue under chemotactic guidance,[43,44] the convenient and minimally-invasive approaches of systemic intravenous or intraperitoneal administration might be therapeutically

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advantageous, compared with the more invasive approaches of local intraventricular or IT administration, especially in very unstable newborn infants who may not tolerate more invasive local injection of stem cells. However, systemically transplanted MSCs might have limitations in crossing the blood brain barrier[17] and could also be retained in other organs, such as the lungs, liver, spleen, and kidneys.[45] Thus, better therapeutic efficacy was observed with local administration of MSCs, compared to four- to five-fold higher doses delivered by systemic injection, in newborn animal models of IVH[17] and BPD.[39] Moreover, because the anterior fontanelle is open in newborn infants, and all preterm infants at high risk for BPD are already intubated to receive ventilator support, further invasive procedures would not be necessary for local delivery of stem cells in these neonates. Based on this preclinical evidence, MSCs were delivered intratracheally or intraventricularly in the first phase I clinical trials of BPD[8] and IVH,[46] respectively.

The right timing

Determining the optimal timing for MSC transplantation is another major issue that remains to be clarified for future clinical translation. For BPD, Pierro, et al.[11] found that administration of MSCs or conditioned media not only at P4 for prevention, but also at P14 for rescue approaches prevented and rescued hyperoxic alveolar growth arrest. However, significant attenuation of hyperoxic lung injuries with MSC transplantation was observed only early (P3) but not late (P10), and no synergies were observed with combined early and late MSC transplantation in the study by Chang, et al.[12] In the study by van Haaften, et al.,[47] MSC transplantation at P4 for prevention, but not at P14 for regeneration, significantly attenuated neonatal hyperoxic lung injuries. Collectively, these data suggest that the therapeutic time window for MSC transplantation for BPD is narrow. Thus, in a clinical setting, early identification of preterm infants at the highest risk for developing BPD and transplantation of MSCs as early as possible within the first few postnatal days seem favorable over later administration in preterm infants with established BPD.

The therapeutic time window of MSCs for neonatal brain injury shows wide variability, ranging from the first hours to 10 days after the insult,[7,48-51] and this wide variation might be attributable to differences in animal models and the severity of brain insult. As the neuroprotective effects of MSCs might be mediated primarily by their paracrine anti-inflammatory and antiapoptotic properties,[7] MSCs must be transplanted during the very acute phase after brain injury for their neuroprotection to be effective. In our recent study conducted to optimize the timing of MSC transplantation for severe IVH,[52] intraventricular transplantation of MSCs early at 2 days, but not at 7 days, after induction of severe IVH significantly attenuated the development of PHH; impaired behavioral function tests; increased apoptosis, astrogliosis, and inflammatory responses; and reduced corpus callosum thickness and brain myelination associated with IVH. This phenomenon was also observed in other disease models, including murine graft-versus-host disease models and experimental autoimmune encephalomyelitis models.[49,53] Overall, the therapeutic time window for stem cell therapy might be narrow; thus, transplantation of MSCs as close as possible to the time of brain insult might provide better therapeutic outcomes. Further studies will be necessary to clarify this.

The right dose

In light of the preclinical data showing wide variation in doses and the resultant therapeutic efficacy according to the route of stem cell administration in animal models of BPD,[13,39] HIE[49] and IVH,[17] the injury site and route of administration seem to be two major determinants of the optimal dose for MSC transplantation. In light of preclinical evidence showing dose-dependent protection with MSC transplantation,[13,49] we tested the safety and feasibility of a single IT transplantation of 1×10^7 cells/kg for low dose or 2×10^7 cells/kg for high dose in our pioneering phase I clincical trial for BPD[8] and a single intraventricular transplatation of 5×10^6 cells/kg for low dose or 1×10^7 cells/kg for high dose in a phase I clinical trial for severe IVH;[46] no dose-limiting toxicity or serious adverse events were observed with either dose. Taken together, these findings suggest that injury site and route of administration are major determinants for the optimal dose of MSCs in transplantation. Based on these findings, further preclinical clinical studies to determine the optimal dose of MSCs for maximal clinical benefit in newborn infants affected by currently intractable and devastating disorders are anticipated.

Safety of MSC transplantation

Considering the early postnatal age of newborn infants who are potential candidates for stem cell therapy, a longitudinal study showing not only therapeutic efficacy, but also both short- and long-term safety in an animal model is essential for successful clinical translation. Due to its short life span, a rodent model is most suitable because both short- and long-term research is feasible in a short time frame. In addition to the persistent protective effects of intranasal transplantation of MSCs against hypoxic ischemic brain injuries in newborn mice up to 14 months after transplant, no abnormalities including neoplasia were observed in the nasal turbinates, brain, or other organs.[54] Similarly, the protective and beneficial effects of IT transplantation of MSCs were sustained without any long-term adverse effects, such as tumorigenicity, in rats up to 70 days[10] or 6 months of age,[11] comparable to human adolescence and mid-adulthood, respectively. Furthermore, in our previous phase I clinical trial,[8] IT transplantation of MSCs was safe and feasible, and no adverse outcomes including tumorigenicity have been reported in follow up of these infants until 2 years of corrected age and in more than 350 clinical studies of MSC transplantation conducted worldwide. The absence of long-term adverse effects including tumorigenicity after MSC transplantation mi-

ght be attributable to the absent engraftment of the transplanted MSCs;[11] thus, they exert their therapeutic function by a "hit and run" mechanism.[55] Overall, the preclinical data indicating the sustained protective effects of MSC transplantation without any short- or long-term adverse effects warrant the translation of MSC transplantation into clinical trials for treatment of intractable disorders in newborn infants.

CONCLUSION

Stem cell therapy for currently intractable and devastating neonatal disorders, such as BPD, HIE, and IVH, might be a potential paradigm shift in neonatal medicine. Exciting progress in preclinical and clinical studies has brought human stem cell therapy for newborn infants one step closer to clinical translation. However, the resolution of several issues, such as safety, up-scaling of manufacturing processes, standardization methods, clinical indications, timing, dosage, and a better understanding of protective mechanisms, are required to permit safe clinical translation of stem cell therapy for newborn infants in the near future. It is essential to proceed step-by-step, rather than in haste, with relentless efforts to overcome these obstacles and make stem cell therapy the next breakthrough in the therapy of these intractable neonatal disorders.

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