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Stem Cell Therapy for Bronchopulmonary Dysplasia: Bench to Bedside Translation

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Bronchopulmonary dysplasia (BPD), a chronic lung disease affecting very premature infants, is a major cause of mortality and long-term morbidities despite of current progress in neonatal intensive care medicine. Though there has not been any effective treatment or preventive strategy for BPD, recent stem cell research seems to support the assumption that stem cell therapy could be a promising and novel therapeutic modality for attenuating BPD severity. This review summarizes the recent advances in stem cell research for treating BPD. In particular, we focused on the preclinical data about stem cell transplantation to improve the lung injury using animal models of neonatal BPD. These translational research provided the data related with the safety issue, optimal type of stem cells, optimal timing, route, and dose of cell transplantation, and potency marker of cells as a therapeutic agent. Those are essential subjects for the approval and clinical translation. In addition, the successful phase I clinical trial results of stem cell therapies for BPD are also discussed.

Keywords: Bronchopulmonary Dysplasia; Cell Transplantation; Mesenchymal Stem Cells; Infant, Premature

INTRODUCTION

Bronchopulmonary dysplasia (BPD) is a chronic lung disease that usually occurs in premature infants receiving prolonged oxygen supplementation and ventilator support. The risk of developing BPD correlates with the extent of immaturity (1). Recent improvements in the survival of very preterm infants through advances in neonatal intensive-care medicine have, therefore, made the task of protecting the extremely immature lungs against BPD increasingly challenging. BPD remains an important cause of mortality and long-term respiratory morbidities such as airway hyperreactivity, poor lung function, and low exercise capacity (2-6). In addition, neurologic morbidities such as developmental delay and cerebral palsy (7) are also common. The histopathological characteristics of BPD include impaired alveolarization and interstitial fibrosis (8, 9). Prolonged oxygen exposure of newborn rat pups results in decreased alveolarization and increased lung fibrosis, thereby simulating the histopathology of human BPD (9, 10). Inflammatory responses are believed to play critical roles in the lung injury process leading to the development of BPD (1). Currently, no effective treatments beyond supportive therapies are available for BPD. Therefore, development of new therapeutic modalities to improve the prognosis of BPD in preterm infants is an urgent priority.

Recently, current literature has shown that the exogenous administration of stem cells significantly attenuated neonatal hyperoxic lung injuries (11-18). These findings suggest that stem cell transplantation might be a new and promising therapeutic modality for the treatment of BPD. In this review, we summarize the recent advances in stem cell research for treatment of BPD. In particular, we focus on the preclinical data regarding the important issues for clinical translation such as the optimal cell type, route, dose, and timing of stem cell therapy. Furthermore, the successful phase I clinical trial results of stem cell therapies for BPD are discussed.

PRECLINICAL RESEARCH DATA

Determining the optimal cell type

Among the various stem cells, the selection of a single appropriate stem cell that ultimately exhibits the best therapeutic efficacy in protecting against BPD is a difficult challenge. Embryonic stem cells are pluripotent cells capable of generating all cell types from three germ layers. However, the high tumorige-

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nicity and ethical concerns of destroying embryos for their acquisition have limited their availability for research and clinical applications (19).

Mesenchymal stem cells (MSCs) are the most extensively examined cell type used in experimental models of BPD (13-18, 20). MSCs are broadly distributed in the body, and could be isolated from adult tissues such as the bone marrow, adipose tissue, and gestational tissues such as the placenta, Wharton's jelly, and umbilical cord blood (UCB). The umbilical cord and placenta are medical wastes that are usually discarded at birth, and therefore, MSCs obtained from gestational tissues seem to be particularly attractive (21). In addition to their easy attainability, MSCs derived from gestational tissues showed less antigenicity (21), and higher proliferation capacity and paracrine potency compared with adult tissue-derived MSCs (22). Even within the same adult tissue origin, donor age negatively impacted the expansion and differentiation potential of the MSCs (23, 24). Collectively, these findings suggest that MSCs derived from post-partum associated tissues such as UCB or Wharton's jelly might be the optimal cell source for future clinical applications, in protecting premature infants against BPD.

Therapeutic potential and protective mechanisms of MSCs for BPD

The therapeutic efficacy of MSCs has been tested in the hyperoxia-induced neonatal rodent or murine model of BPD, and was reported to improve survival, and suppress oxidative stress and inflammation (11, 13-18, 20). In addition, it attenuated the impaired alveolar growth, lung vascular injuries, fibrosis, and the associated pulmonary hypertension (11, 13-18, 20). These findings support the assumption that stem cell transplantation might be a promising novel therapeutic approach for BPD.

The beneficial effects were initially ascribed to the transdifferentiation of MSCs into lung parenchymal cells such as type II pneumocytes (11, 25). However, this event rarely occurs in vivo (11). The low rate of in vivo engraftment and differentiation into lung tissue suggests that the therapeutic effects of stem cell transplantation might not be primarily mediated by regeneration. An equal or better therapeutic efficacy in preventing or reversing established BPD was observed with MSC-conditioned media compared with MSC (13, 26, 27). More recently, Lee et al. (28) have reported that microvesicles released by MSC exosomes are the major paracrine anti-inflammatory and therapeutic mediators of MSCs in hypoxia-induced pulmonary hypertension. Collectively, these findings suggest that the protective effects of stem cell transplantation might be predominantly mediated by paracrine, rather than regenerative, mechanisms. The use of MSC secretomes rather than stem cells could be an exciting, promising new therapeutic approach for BPD, especially since it circumvents the theoretical concerns associated with live cell treatments, such as tumor formation.

The specific humoral substances secreted by the transplanted MSCs that are responsible for the protective paracrine activity have not yet been elucidated. In our previous experiments, we observed that significantly reduced levels of growth factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) were significantly improved with MSCs transplantation (17). Moreover, the knockdown of VEGF secretion by the MSCs using transfection with small interfering RNA specific for human VEGF abolished the protective effects of MSCs in hyperoxic lung injury (18). These protective effects included the attenuation of impaired alveolarization and angiogenesis, reduction in apoptotic cells and alveolar macrophages, and downregulation of proinflammatory cytokine levels (18). Overall, these findings suggest that growth factors such as VEGF, which are secreted by the transplanted MSCs, are critical paracrine factors that mediate the protective effects of MSCs against hyperoxic lung injuries.

Determining the optimal route, dose, and timing of MSC transplantation

Determining the optimal route of MSC transplantation is a critical issue that needs to be resolved for future successful clinical translation of stem cell therapies for protection against BPD. Injured lungs produce chemotactic factors that cause MSCs to proliferate and migrate toward the injury (28). Furthermore, systemically administered MSCs have been shown to home and localize to an injured lung (29). Local intratracheal transplantation of MSCs at four times lesser doses produced more effective engraftment of donor cells and attenuation of hyperoxia-induced lung injury than with systemic intravenous or intraperitoneal administration (11). These findings suggest that the local intratracheal rather than systemic intravenous or intraperitoneal transplantation of MSCs might be an optimal route of delivery for treating premature infants with BPD.

Determination of the optimal dose of MSCs for transplantation is another important issue that needs to be addressed for successful clinical translation. In our previous study (15), we tested the therapeutic efficacy of three different doses of human UCB-derived MSCs (5×10^3, 5×10^4, and 5×10^5 cells) administered intratracheally to hyperoxic newborn rat pups (average weight, 8 g) at postnatal day (P) 5. The intratracheal transplantation of human UCB-derived MSCs attenuated the symptoms associated with hyperoxia-induced lung injury, such as decreased alveolarization, in a dose-dependent manner. The dose of 5×10^5 cells provided the best protection, while at least 5×10^4 cells were necessary for effective anti-inflammatory, anti-fibrotic, and anti-oxidative activity. In the light of these findings, further studies to determine the optimal dose of human UCB derived MSCs for potential clinical benefit in human preterm neonates are planned.

While the therapeutic efficacy of MSC transplantation in BPD

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has already been shown (11), the optimal timing of administration is another critical issue that remains to be established. Therefore, we attempted to determine the optimal timing by comparing the therapeutic efficacy of early (at P3) versus late (at P10) intratracheal transplantation of MSCs (17). We observed that hyperoxia-induced lung injuries such as impaired alveolarization, increased apoptosis, oxidative stress, inflammation, and fibrosis, as well as reduced VEGF and HGF levels were significantly attenuated with early but not late transplantation. These findings suggest that the therapeutic time window of MSC transplantation for BPD may be narrow during the early but not the late phase of inflammatory responses.

Long-term safety and outcome of MSC transplantation

Peirro et al. (20) reported that both human umbilical cord-derived perivascular cells and MSCs exerted short- and long-term (6 months) therapeutic benefits including persistent improvement in lung structure and exercise capacity, despite the low engraftment of cells. Moreover, no tumor formation was observed, and the beneficial effects of intratracheal transplantation of MSCs in neonatal hyperoxic lung injuries were evident at P5. These beneficial effects, which included improved alveolar and vascular growth, were sustained for a prolonged recovery period without any long-term adverse effects up to P70 (16). Overall, these findings support the assumption that transplantation of MSCs to prevent or treat BPD in premature infants at a critical early time point might modify and improve the long-term respiratory morbidities of BPD.

PHASE I CLINICAL TRIAL OF MSC FOR BPD

The safety and feasibility of transplanting allogeneic human UCB-derived MSCs in preterm infants was assessed. Intratracheal transplantation of MSCs was performed in 9 preterm infants (3 received 1×10^7 cells/kg and 6 received 2×10^7 cells/kg) who had a very high risk for developing BPD. The infants in this phase I clinical study had a mean gestational age of 25.3 ± 0.9 weeks, a mean birth weight of 793 ± 127 g and a mean birth age of 10.4 ± 2.6 days (30). The transplantation was well tolerated, without any serious adverse events or dose-limiting toxicity. Tracheal aspirate cytokine levels at day 7 were significantly reduced compared with the baseline levels. Moreover, BPD severity which classified as mild, moderate, and severe according to the consensus of NICHD workshop (31), was significantly lower in the transplant recipients compared with the gestational age, body weight, and respiratory severity-matched control group. Overall, these findings suggest that intratracheal transplantation of allogeneic human UCB-derived MSCs in very preterm infants at the highest risk for developing BPD is safe and feasible. A long-term follow-up safety study (NCT01632475) on MSC-treated preterm infants and a phase II double-blind randomized controlled trial to assess the therapeutic efficacy (NCT 01828957) are currently underway.

CONCLUSIONS

In recent years, we have broadened our knowledge and understanding of stem cell therapy for neonatal lung injury. Contributions to this advancement include the various translational research studies supporting the therapeutic potential, safety profile, optimal route, optimal timing, optimal dose, and potential efficacy marker of stem cell therapies for BPD. Moreover, the first phase I clinical trial of MSC transplantation for BPD was conducted successfully, proving its safety and feasibility in the preterm infants. This progress has moved human stem cell therapy for BPD one step closer to clinical translation (Tables 1, 2). We are currently conducting two essential studies to be introduced clinically. The first is a phase II clinical trial to assess the therapeutic efficacy (NCT01828957), and the second is a long-term follow-up safety assessment study of the MSC trans-

Table 1. Progress of translational research of MSC for neonatal BPD

Translational study of MSC for the hyperoxia induced lung injury in the newborn rats					
Source	Transplantation: Timing	Transplantation: Dose	Transplantation: Route	Outcome	Reference
UCB	P5	5×10^5 for IT/2×10^6 for IP	IT/IP	Optimal route: IT > IP improved hyperoxic lung injury	11
UCB	P5	5×10^3/5×10^4/5×10^5	IT	Dose-dependently improved hyperoxic lung injury	15
UCB	P3/P10/P3+10	5×10^5	IT	Optimal timing; Early > Late improved hyperoxic lung injury	17
UCB	P5	5×10^5	IT	No visible mass lesion and persistent improved alveolarization and inflammation in the lungs until P70	16
BM	P4	1×10^5	IT	Attenuated alveolar and vascualr injury and reduced pulmonary hyptertension	14
BM	P4	5×10^4	IV	Reduced alveolar loss and lung inflammation	26
UCB	P4	3×10^5	IT	No tumor lesion and persistent improved alveolarization with improved exercise capacity until 6 months	20
BM	P9	2×10^6	IT	Persistent mproved alveolarization and lung angiogenesis until P100	32

MSC, mesenchymal stem cells; BPD, bronchopulmonary dysplasia; UCB, umbilical cord blood; BM, bone marrow; P, postnatal day; IT, intratracheal; IP, intraperitoneal; IV, intravenous.

Table 2. Clinical research of MSC for neonatal BPD

Clinical study for the prevention of BPD in the premature infafnts				
Phase	Year	ClinicalTrials.gov identifier	Status	Reference
Phase 1	2011	NCT01297205	Completed	30
Follow-up	2012	NCT01632475, NCT02023788	Ongoing	
Phase 2	2013	NCT01828957	Ongoing	
Follow-up	2013	NCT01897987	Ongoing	

MSC, mesenchymal stem cells; BPD, bronchopulmonary dysplasia.

plant recipients (NCT01897987). Conditional approval of clinical use of MSC might be anticipated cautiously after the completeion of the phase II clinical trial with favorable outcome.

DISCLOSURE

Samsung Medical Center and MEDIPOST Co, Ltd have issued or filed patents for “Method of treating lung diseases using cells separated or proliferated from umbilical cord blood” under Yun Sil Chang, Won Soon Park, and Yoon Sun Yang (not affiliated with this article) (application PCT/KR2007/000535).

AUTHOR CONTRIBUTION

All authors participated in writing and revision and agreed to final manuscript.

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