Can We Cure Bronchopulmonary Dysplasia?

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Bronchopulmonary Dysplasia–the Ransom of Success

Fifty years after Dr Northway's original description of bronchopulmonary dysplasia (BPD), the disease still lacks a cure. This can be interpreted as a failure of progress, or conversely, as a ransom of our success in improving the survival of ever more preterm infants. Consequently, preterm infants at risk for BPD today have more immature lungs than in the past, making the task of preventing or rescuing lung damage more daunting. Classic pharmacologic therapies and past technologic advances appear to have had only minor impact on disease progression. Clearly, dramatic improvements can only be expected from paradigm-shifting approaches that have similar impact as surfactant therapy for respiratory distress syndrome in the 1990s.

Cell therapies may represent such a game changer. Cells have pleiotropic properties that are distinct from those of classic drug therapies. "Part drug and part device, cells sense diverse signals, move to specific sites in the body, integrate inputs to make decisions, and execute complex response behaviors—all in the context of a specific tissue environment."[1] Potentially, these exciting properties can be harnessed for lung regeneration, especially in the setting of severe BPD. This review will briefly discuss current preclinical and clinical evidence underlying the promise of cell therapies for BPD and highlight how much more needs to be learned about the underlying mechanism(s) of action of therapeutic cells and their manufacturing process to ensure timely, yet safe, clinical translation of this potential paradigm-shifting approach.

Cell-Based Therapies–Smart Pharmacies Capable of Multitasking

Since the original description of stem cells in 1960,[2] recent insights into stem cell biology have unraveled the therapeutic potential of stem cells and propelled regenerative medicine as the next paradigm shift. Stem cells have the capability to self-renew and to differentiate into any cell (totipotent; eg, zygote), any cell of the 3 germ layers (pluripotent; eg, embryonic stem cells), certain cell types (multipotent; eg, hematopietic stem cells), or specific cell types (bi- and unipotent; eg, hepatocytes or alveolar epithelial cells). These properties were thought to underlie the capability of stem cells in restoring organ integrity after injury and formed the premise for exploring the therapeutic potential of cell therapy in experimental neonatal lung injury.

Among the many stem cells with repair potential that have been studied over the past decade, mesenchymal stromal cells (MSCs) have attracted the most attention because of their ease of isolation, culture, and expansion. First, isolated from the bone marrow, MSCs exist in almost all organs. MSCs are fibroblast-like multipotent cells that can give rise to bone, fat, and cartilage, adhere to plastic, and express certain cell surface markers, as defined by minimal criteria.[3] Even though the characterization of MSCs is still evolving, the potent pleiotropic repair potential of MSCs combined with their low immunogenic potential, make them the ideal cell-based therapy for a multifactorial disease such as BPD. Promising preclinical investigations have already led to the initiation of clinical trials for various adult and pediatric diseases, including BPD.

Preclinical Evidence for MSC Therapy in BPD

Proof-of-concept experiments with bone marrow-derived MSCs demonstrated prevention of oxygen-induced arrested alveolar and lung vascular development in neonatal rodents.[4,5] Numerous investigators subsequently confirmed the ability of MSCs to prevent neonatal lung injury in these same models, via single intratracheal, intravenous, or intraperitoneal injection, whereas intranasal delivery did not result in therapeutic benefit (reviewed in Mobius and Thebaud[6]). Human umbilical cord tissue and cord blood have been proposed as a clinically relevant source of cells for MSC therapy. Experiments in the same neonatal rodent model showed efficacy in lung injury prevention and rescue, attenuation of pulmonary hypertension, as well as safety up to 6 months post-treatment.[7,8]

Besides the well-known potent anti-inflammatory effects of these cells, MSCs also demonstrated antifibrotic, antiapoptotic, antioxidative, and proangiogenic properties, as well as lung growth promoting effects. It is now clear that MSCs do not

BPD	Bronchopulmonary dysplasia
EVs	Extracellular vesicles
MEX	MSC-derived exosomes
MSCs	Mesenchymal stromal cells

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engraft in the target organ and that their therapeutic benefit is mediated via so-called "paracrine" activity (reviewed in Fung and Thebaud[9]). Indeed, very few cells can be found in the lung within days after airway or intravenous delivery, consistent with earlier findings in the cardiac and brain literature. Accordingly, cell-free conditioned media confers similar (and in some studies better) lung protection, even after a single injection,[4] raising intriguing questions about the bioactive molecules at play. Recent findings suggest that MSCs communicate and release bioactive compounds via extracellular vesicles (EVs) or exosomes (as reviewed in Kourembanas[10]).

EVs, and particularly the type secreted through the endocytic-exosomal pathway (exosomes), were originally assumed to represent a method for the cell to jettison unwanted moieties. During their biogenesis, EVs or exosomes engulf part of their parental cell, becoming enriched in an array of bioactive cargo that has been reported to include genetic information (such as small noncoding RNAs), free fatty acids, surface receptors and protein.[10,11] In turn, the secretion of these signaling vectors to the extracellular environment represents an effective method of cell-to-cell communication.

Interest in the therapeutic potential of exosomes has exploded in recent years. MSC-derived exosomes (MEX) have demonstrated beneficial effects in a number of preclinical models.[10] Such diverse and potent effects following MEX treatment provide an exciting opportunity to develop an innovative therapy for multifactorial diseases such as BPD. However, a full realization of the therapeutic capacity of MEX has been hampered by the absence of standardization in exosome isolation/purification. Often, crude exosome isolation techniques (such as ultracentrifugation), and poor analytical characterization obfuscates the therapeutic impact of MEX, co-contaminating preparations with non-exosomal material and impairing bioavailability.[12] Thus, although stem cell-based therapies (such as MEX) may represent the next major breakthrough for diseases of the newborn, several challenges such as difficulties in assessing exosome potency and scaling toward industry-scale production remain when considering the transition to clinical development.[13]

Overall, these observations open exciting therapeutic avenues for established BPD and perhaps other complications of extreme prematurity. Indeed, separate studies showed therapeutic benefit of MSCs in experimental intraventricular hemorrhage, necrotizing enterocolitis, and neonatal sepsis.[14] Thus, unlike postnatal steroids, MSCs exert their anti-inflammatory properties without adverse effects on brain development and host-defense, at least in experimental models. These promising findings already prompted the first clinical trials of MSCs for BPD.

Initial Clinical Trials of MSCs for BPD

A first phase I dose-escalation study with allogeneic cord blood-derived MSCs in preterm neonates at risk of developing BPD enrolled 9 extremely preterm neonates (23-29 weeks gestation, birth weight of 500 and 1250 g) to receive a single intratracheal injection of MSCs within 5-14 days of life if still on mechanical ventilation.[15] The procedure was feasible, and no serious adverse events were reported within 6 hours of cell administration. The 2-year follow-up study of these 9 preterm infants shows no adverse effects on growth, respiratory and neurodevelopmental outcomes.[16] These studies provide some indication on feasibility and short-term safety in a small number of patients but are far too limited to generalize about safety or efficacy. Results of a completed phase I study with a similar design (NCT02381366) and a phase II study (NCT01828957) are pending. Even though much more needs to be learned about the biology of MSCs, well-designed early phase clinical trials are warranted to cautiously advance our knowledge about the clinical use of these innovative therapies. All patients receiving cell therapy should be enrolled in a registry to ensure adequate safety monitoring.

Future Considerations

Although MSCs have attracted most of the attention, other cell products have shown promising results in experimental neonatal lung injury. Human amnion epithelial cells exert similar effects as MSCs and prevent lung injury in several animal models, ranging from fetal sheep to neonatal and adult rodents (reviewed in Zhu et al[17]). These cells are currently in study as a phase I trial in infants at risk for developing BPD. Endothelial progenitor cells also promote lung growth and attenuate pulmonary hypertension with safety at 10 months post-treatment.[18] More recently, exogenous alveolar macrophage therapy has been proposed as an alternate cell product.[19] Direct side-by-side comparisons may need to be performed to determine if one cell is more effective over another.

As the benefits of MSC and perhaps other repair cells are due to their paracrine effects, there is a need to study the potential benefit of "cell therapies without the cell." The realization that MSCs release their therapeutic factors packaged in exosomes may further facilitate the manufacture of cell-free products derived from MSC. Cell-free therapy may alleviate the risk of tumor formation, but the capability of MSCs to interact with the local environment and adapt the release of therapeutic factors may be lost. Further studies need to identify the best therapeutic option depending on clinical indications.

Any aspect during the cell manufacturing process (from initial source selection, cell isolation, and expansion to freezing and thawing until administration) will affect the repair potential of the cells.[20] Detailed knowledge about the manufacturing process and the development of reliable potency assays will be crucial to assess the quality of the cell product and adequate interpretation of study results.

In parallel to early phase clinical trials, continued discovery research at the bench remains critical to improve our understanding about the mechanism of action of repair cells. Likewise, a better characterization of resident stem cells during health and changes with disease may allow the development of superior cell products with enhanced repair capabilities.[21]

Conclusions

Cell-based therapies may represent a paradigm shift in the treatment of BPD. Preclinical studies show promising therapeutic benefit in animal models and have led to early phase clinical studies to examine the feasibility and safety of MSCs in preterm infants at risk of developing BPD. Yet, much more needs to be learned about the biology of stem cells, their mechanism of action, and manufacturing processes to deliver safe and effective cell products.[22] It is critical that the translation of cell therapies is based on robust preclinical evidence and well-designed clinical trials. Like with any disruptive technology, major refinements will need to be made in coming years to fulfill the exciting potential of cell-based therapies. ■

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References

1. Fischbach MA, Bluestone JA, Lim WA. Cell-based therapeutics: the next pillar of medicine. Sci Transl Med 2013;5:179-7.
2. McCulloch EA, Till JE. The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice. Radiat Res 1960;13:115-25.
3. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-7.
4. Aslam M, Baveja R, Liang OD, Fernandez-Gonzalez A, Lee C, Mitsialis SA, et al. Bone marrow stromal cells attenuate lung injury in a murine model of neonatal chronic lung disease. Am J Respir Crit Care Med 2009;180:1122-30.
5. van Haaften T, Byrne R, Bonnet S, Rochefort GY, Akabutu J, Bouchentouf M, et al. Airway delivery of mesenchymal stem cells prevents arrested alveolar growth in neonatal lung injury in rats. Am J Respir Crit Care Med 2009;180:1131-42.
6. Mobius MA, Thebaud B. Cell therapy for bronchopulmonary dysplasia: promises and perils. Paediatr Respir Rev 2016;20:33-41.
7. Chang YS, Choi SJ, Ahn SY, Sung DK, Sung SI, Yoo HS, et al. Timing of umbilical cord blood derived mesenchymal stem cells transplantation determines therapeutic efficacy in the neonatal hyperoxic lung injury. PLoS ONE 2013;8:e52419.
8. Pierro M, Ionescu L, Montemurro T, Vadivel A, Weissmann G, Oudit G, et al. Short-term, long-term and paracrine effect of human umbilical cord-derived stem cells in lung injury prevention and repair in experimental bronchopulmonary dysplasia. Thorax 2013;68:475-84.
9. Fung ME, Thebaud B. Stem cell-based therapy for neonatal lung disease: it is in the juice. Pediatr Res 2014;75:2-7.
10. Kourembanas S. Exosomes: vehicles of intercellular signaling, biomarkers, and vectors of cell therapy. Annu Rev Physiol 2015;77:13-27.
11. van der Pol E, Boing AN, Harrison P, Sturk A, Nieuwland R. Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol Rev 2012;64:676-705.
12. Witwer KW, Buzas EI, Bemis LT, Bora A, Lasser C, Lotvall J, et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. J Extracell Vesicles 2013;2:1-25.
13. Lener T, Gimona M, Aigner L, Borger V, Buzas E, Camussi G, et al. Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. J Extracell Vesicles 2015;4:30087.
14. Zhu Y, Xu L, Collins JJP, Vadivel A, Cyr-Depauw C, Zhong S, et al. Human umbilical cord mesenchymal stromal cells improve survival and bacterial clearance in neonatal sepsis in rats. Stem Cells Dev 2017;26:1054-64.
15. Chang YS, Ahn SY, Yoo HS, Sung SI, Choi SJ, Oh WI, et al. Mesenchymal stem cells for bronchopulmonary dysplasia: phase 1 dose-escalation clinical trial. J Pediatr 2014;164:966-72, e6.
16. Ahn SY, Chang YS, Kim JH, Sung SI, Park WS. Two-year follow-up outcomes of premature infants enrolled in the phase I trial of mesenchymal stem cells transplantation for bronchopulmonary dysplasia. J Pediatr 2017;185:49-54.e2.
17. Zhu D, Wallace EM, Lim R. Cell-based therapies for the preterm infant. Cytotherapy 2014;16:1614-28.
18. Alphonse RS, Vadivel A, Fung M, Shelley WC, Critser PJ, Ionescu L, et al. Existence, functional impairment, and lung repair potential of endothelial colony-forming cells in oxygen-induced arrested alveolar growth. Circulation 2014;129:2144-57.
19. Litvack ML, Wigle TJ, Lee J, Wang J, Ackerley C, Grunebaum E, et al. Alveolar-like stem cell-derived Myb(-) macrophages promote recovery and survival in airway disease. Am J Respir Crit Care Med 2016;193:1219-29.
20. Sharma RR, Pollock K, Hubel A, McKenna D. Mesenchymal stem or stromal cells: a review of clinical applications and manufacturing practices. Transfusion 2014;54:1418-37.
21. Mobius MA, Thebaud B. Bronchopulmonary dysplasia — where have all the stem cells gone? Origin and (potential) function of resident lung stem cells. Chest 2017, in press.
22. Daley GQ. Polar extremes in the clinical use of stem cells. N Engl J Med 2017;376:1075-7.