Regenerative medicine - From stem cell biology to clinical trials for pediatric heart failure

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1. Introduction

Progressive heart failure in children and young adults is a serious problem, with an overall mortality of 7% in the United States [1]. In the past, the vast majority of children with heart failure had either cardiomyopathy or myocarditis, however, with improved surgical survival of high risk neonates with congenital heart disease, the incidence of heart failure due to structural heart disease has increased dramatically. There are critical differences in the response to injury between the young and the mature heart the potential for regeneration is greater in the former, in comparison to the more limited response in the older adult heart. As well, the etiologies of pediatric heart failure are less likely to lead to large areas of fibrosis, which is typical of the cardiomyopathy seen after myocardial infarction in adults [2]. Notably, pressure overload in the right ventricle in pediatric hearts results in a 3-fold increase in cardiac progenitors and suggests that an adaptive response is present in children, yet this innate regeneration is not sufficient to overcome the challenges of severe heart disease [3]. Genetic, environmental, or metabolic challenges can accelerate progressive heart failure despite the best supportive surgical and medical treatment. Unfortunately, the supply of available pediatric transplant donor hearts is a major limitation and even patients who undergo successful transplant trade cardiac dysfunction for the life-long complications of immunosuppression. Therefore, to provide new innovative strategies for these patients, regenerative technologies need to be fully evaluated as a transformative strategy. There is a rapidly growing list of cell-based technologies that could be re-purposed for pediatric heart disease applications [4], with appropriate concerns for safety in this unique population. The future of cardiac regenerative medicine for pediatrics is being shaped based on emerging clinical trials and rapid advances in our knowledge of cardiac progenitor cell biology. This review highlights the current knowledge in the new field of cell-based regenerative therapies and the potential these strategies offer children with severe heart disease.

2. Cell-based Therapy to Improve Cardiac Function in Adult Heart Disease

With more than a decade of dedicated effort, the science of cardiac regenerative medicine has provided the medical community with substantial data on the advantages and limitations of cell-based therapy, e.g., bone marrow derived stem cells (BMSC) in adults with ischemic heart disease. Having established a firm foundation focused on important safety profiles, clinical trials have demonstrated no evidence of increased arrhythmogenicity, acute cardiac toxicity, or tumor formation [5–10]. Depending on the characteristics of the patient population studied and the cell-type or delivery method used, cardiac performance has improved, but so far with only modest efficacy. Therefore, the focus on next-generation products and delivery strategies will need to continue to improve these early results. Of note, one of the most powerful associations in these studies has been the age of the patient as younger patients are more likely to have superior outcomes following cell-based delivery [11,12]. When cell-based therapies have been scrutinized in the laboratory, it is becoming apparent that the mechanisms by which they enhance regeneration are largely through a paracrine mediated augmentation of the limited underlying innate regenerative response of the myocardium, rather than by long-term survival and integration of existing cells [13–16]. Indeed, studies have shown that the majority of stem cells will be gone within a month of administration, despite their effect on improving cardiac function [17]. Therefore, providing these paracrine mediated effects at a time which will provide maximal benefit may be one of the predominant determinants that predicts optimal outcomes. Alternative modalities, which may eventually have application for the pediatric heart failure population, include the use of epicardial bioengineered patches, which can be used for the longer-term delivery of specific paracrine factors directly to injured myocardium [18].

3. Cardiac Regenerative Medicine for Patients with Congenital Heart Disease (CHD)

On the basis of early results in adult patients, the application of cell-based therapies to pediatric patients is primed to open new therapeutic
paradigms to augment the regenerative potential of the immature heart. Experience with cell-based therapy in children with severe heart disease is quite limited compared to the adult experience, and to date no large clinical trials have been reported using cell-based therapy. Many early reports have used bone marrow derived cells administered via intracoronary infusion, in patients with either single ventricles or dilated cardiomyopathy.

One of the initial examples of cell-based therapy in a pediatric patient was a case report in a patient with dilated cardiomyopathy, reported in 2009 [19]. An intracoronary injection of autologous BM-MNCs (bone marrow-mononuclear cells) was associated in a doubling of LV ejection fraction from the 20% range to 40% range after a period of 6 months. Another case report of cell-based infusion was in an 11-month-old patient with congenital heart disease. There were no early complications or adverse events at 3 months follow-up. Magnetic resonance imaging showed an improvement of the calculated ejection fraction of the systemic right ventricle, from 22% to 44%, along with a reduction of end-diastolic and end-systolic volumes, indicating reverse remodeling. The brain natriuretic peptide levels decreased from 2500 pg/ml to 132 pg/ml [20]. In a larger series, bone marrow-derived mononuclear cells were delivered intracoronary in 9 children with end-stage heart failure, 6 of whom suffered from dilated cardiomyopathy and 3 from congenital heart disease. Starting with a bone marrow aspirate, autologous cells were processed and infused on the same day. A stop-flow technique was used for intracoronary delivery and was reported to cause transient ST-segment changes. There were no procedure-related unexpected adverse events. Three patients with dilated cardiomyopathy showed improvement in brain natriuretic peptide serum levels and in ejection fraction throughout intermediate-term follow-up [21]. These studies suggest the potential for a meaningful regenerative response, with low acute risk, after the delivery of autologous mononuclear cells for pediatric heart disease. However, as none of these studies were performed with controls, their findings can only be regarded as the first step.

A Phase I study examining autologous cell-based therapy in congenital heart disease using intracoronary infusions of cardiac derived cells (the TICAP trial) was completed in 2013 [12,22]. In this non-randomized controlled study, 14 consecutive patients with HLHS were prospectively assigned to receive intracoronary cardiac-derived cells cultured from a cardiac biopsy one month after cardiac surgery (n = 7), followed by 7 control patients who received standard care alone. There were no serious adverse events within the 36 month follow-up period. Echocardiography demonstrated an improvement in RVEF compared with the case controls (+8% ± 4.7% vs +2.2% ± 4.3%). Based on these encouraging results, the first randomized-controlled, prospective phase 2 clinical trial is currently being conducted at Okayama University in Japan. The Cardiac Progenitor Cell Infusion to Treat Univentricular Heart Disease (PERSEUS-NCT01829750) trial has been designed to assess the efficacy of intracoronary infusion of cardiac-derived cells (CDCs) in young patients (up to 20 years of age) with univentricular heart disease (hypoplastic left heart syndrome, single right ventricle and single left ventricle). A total of 34 patients will be randomly assigned 1:1 to the treated or control group. Patients included in this study will have had a cardiac biopsy at the time of surgery.

Other ongoing clinical trials using autologous cells in pediatric heart disease include phase I trials at the Mayo Clinic (BM) and at Duke University (NCT01445041). The Mayo Clinic study is using autologous bone marrow derived mononuclear cells to be infused into the coronary circulation of failing Fontan patients with a systemic right ventricle. The primary endpoints will be cardiac performance before and after cell delivery. The Duke study is evaluating the safety and feasibility of collecting and intravenous infusion of autologous umbilical cord blood in newborns with HLHS at the time of cardiac surgery. Endpoints will include neurological improvement and cardiac performance.

In addition to these intracoronary infusion studies, the Mayo Clinic is leading a study with the University of Oklahoma to determine the safety and feasibility of intramyocardial delivery of autologous mononuclear cells in high-risk congenital heart disease patients at the time of a planned Glenn operation for HLHS (NCT01883076). The main goal of this study is to determine the safety profile and feasibility of intramyocardial delivery of autologous mononuclear cells (MNCs) for children with structural heart disease as compared to a parallel observational study (NCT01708863) with identical inclusion and exclusion criteria and prospective follow-up in an open study design. The first case report of direct intramyocardial injection of umbilical cord blood derived MNCs in an infant with HLHS has been published [23]. Umbilical cord blood was collected at the time of delivery, and the MNCs fraction isolated and stored. Cells were then injected directly into the right ventricular myocardium at the time of the Stage II (Glenn shunt) palliation. No adverse events occurred either at the time of infusion or throughout the post-procedural follow-up. Transthoracic echocardiography at 3 months showed improvement in right ventricular systolic function, with an ejection fraction of 50%, increased from 30 to 35% at baseline prior to surgery.

Related to the published intramyocardial delivery strategy, the University of Miami is planning a study (NCT02398604) focused on HLHS pediatric patients to deliver allogeneic mesenchymal stem cells (MSCs), targeting an enrollment of 30 patients. The first 20 patients will receive allogeneic MSCs to determine feasibility and safety. The next 20 HLHS patients will be randomized to the treatment and control arms in a 1:1 ratio, respectively. The major difference between this study and those above is that it will be the first use of allogeneic cells in a pediatric population.

4. Translational Research: Cell-based Emerging Technologies for Cardiac Regeneration in Pediatric Heart Disease

As clinical trials are ramping up in the early stages for pediatric heart disease, pre-clinical studies will become increasingly important to assist in determining valid clinical endpoints for Phase III studies. Given the differences between the etiologies of pediatric and adult heart disease, reviewed above, pre-clinical studies will be required to optimize the matching of regenerative technologies with specific pediatric patient cohorts. Pre-clinical studies [24–27] have begun to specifically test cell-based regenerative products from a wide variety of cell sources including bone marrow, cord blood, peripheral blood and cardiac-derived cells. Beyond the first-generation of cell-based products, there is a growing list of cells, growth factors, and genetic engineering strategies that have been pioneered in adult patients [28]. Given the much more limited number of pediatric patients with heart failure overall, it will be important to learn from these adult studies as we prioritize the next steps for our pediatric patients. Thus, the importance of pre-clinical pediatric model systems to compare cell-based products head to head in the most clinically relevant models.

Current scientific understanding of cardiac regeneration highlights an age-dependent process of cardiomyocyte renewal that is evolutionarily conserved to various degrees across species [29]. The transition from the fetal environment, which is permissive to cardiac regeneration, to the post-natal environment, where regeneration is markedly reduced, will require further study to determine factors, e.g., alterations in oxygen tension, levels of reactive oxygen species (ROS) production and their effects on regenerative gene expression in the mammalian heart [30,31]. This self-renewal appears to be predominately due to existing cardiomyocyte successfully undergoing cytokinesis rather than generation of new cardiomyocytes from a resident stem cell reservoir, as is the case in skeletal muscle [32]. Although this process slows after birth to approximately 1% cellular renewal per year, there is a further decline in late adolescence/early adulthood that suggests that the capacity to reactivate this regeneration may be increased in children compared to older adults [33]. Future regenerative strategies...
should be aligned with three principles: preserving the self-renewal function of the young heart, augmenting basal levels of cardiac self-renewal, and bioengineering additional cardiomyocytes for replacement of tissue that is dysfunctional or even lacking in the case of many congenital heart diseases.

5. Caveats Unique to the Pediatric Population

Although studies have so far, fortunately, demonstrated the general safety of cell infusions or direct intramyocardial injection in children, the sample size in these studies has been quite small. Serious complications that occur with low frequency may not be detected until larger clinical trials are performed, which is a difficult task in the pediatric population. Children treated with such therapies will also be exposed to many decades of potential risk compared to most adults. This could theoretically increase the risk for the development of cell-therapy-related arrhythmias or teratomas/malignancies in our youngest patients. Long-term follow-up has a totally different definition for a 5-year-old than it does for a 65-year-old. Finally, the selection of appropriate controls will be critical, especially given the large variance in indices of cardiac function that can occur naturally in a population of patients with cardiomyopathy or congenital heart disease.

6. Conclusion

Cell-based therapies for pediatric patients with heart failure have enormous potential to usher in a new paradigm of treatment. The success of these therapies in the pediatric population may be enhanced by the lack of large areas of infarct scarred myocardium typical of adults with ischemic heart disease, as well as the enhanced (although still limited) ability of the young heart for innate regeneration. Ongoing clinical and pre-clinical studies will inform our understanding of the basic cellular and molecular biology of these therapies, and help determine the appropriate clinical endpoints, in order to optimize regenerative potential in the pediatric setting. Ultimately, successful regenerative therapies will likely combine multiple components, such as priming pluripotent stem cells for cardiogenesis with chemokines and growth factors, efficiently delivering cells directly into diseased heart muscle to minimize cell loss, augmenting regenerative potential with repeated dosing, and stabilizing the microenvironment with biomaterials for sustainable improvements in cardiac structure and function. Innovation is always debated and never the product of a single attempt, but rather an iterative process of refinement through scientific exploration. The challenge for the field of pediatric heart disease regeneration is to build synergy between clinical practice and discovery science to prioritize the people, processes, and technology with a focus on lifelong healthy outcomes.

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References


