Review paper

Stem cell therapy for single ventricle congenital heart disease – current state and future directions

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Abstract

Hypoplastic left heart syndrome (HLHS) is one of the most complex forms of congenital heart disease, characterized by an underdeveloped left ventricle, outflow tract and aorta. Current surgical and medical treatment for this disease remains palliative. As a result of the multi-step surgery, the right ventricle plays the role of the systemic ventricle, which inevitably leads to its failure. There is an urgent need to develop new treatments to ameliorate the right ventricle failure. Stem cell therapy may represent a new approach to single ventricle pathology. Great numbers of small and large animal studies have proven this therapy to be safe and effective in hypoplastic left heart syndrome. Several clinical trials have been designed to investigate the potential of mesenchymal stem cells in univentricular heart physiology. With increasing evidence, understanding of the mechanism of stem cells' action has shifted from the concept of differentiation into various heart cell types to paracrine activity playing the major role. The secretome of stem cells has been identified as their functional unit. In this review, we present different types of stem cells used in single ventricle diseases in children as well as their preclinical investigations. We also summarize clinical applications of stem cells in children with HLHS.

Key words: hypoplastic left heart syndrome, systemic ventricle, neonatal mesenchymal stem cells, heart failure.

Introduction

The most surgically challenging group of congenital heart diseases comprises those with hypoplastic heart chamber, either right or left. Such patients usually undergo multiple reconstructive procedures to provide sufficient function of the single ventricle which delivers oxygenated blood to the body while deoxygenated blood is passively delivered to the pulmonary circulation. Although pediatric cardiac surgery can correct the cardiac anatomy and physiology to an extent, the patients are always at risk of cardiac failure.

Hypoplastic left heart syndrome (HLHS) is one of the most complex forms of congenital heart disease, characterized by a small, nonfunctional left ventricle (LV) and underdevelopment of the aorta and the aortic and mitral valves [1]. It carries high early mortality and inevitable failure of the right ventricle (RV) working as the systemic ventricle. It is the most common type of single ventricle pathology with the prevalence of 2–3 per 10 000 live births [2–4].

More than 40 years ago comfort care was the only available therapeutic option for children suffering from HLHS. Nowadays the 3-step surgical treatment is well established [1]. During the past years numerous modifications have been made to find a longer lasting and more efficient method of palliation. As the first step, the Norwood procedure aims to relieve systemic outflow tract obstruction and provide nonrestrictive coronary blood flow and nonrestrictive atrial septal defect as well as adequate pulmonary blood flow. It is performed during the first weeks of the child's life. The aim of the second step, bidirectional cavopulmonary anastomosis, is rearranging the vessels and connecting the superior vena cava with the pulmonary artery. It takes place approximately at the age of 6 months. The third and last step procedure, known as the Fontan operation, redirects the remaining desaturated blood from the lower portion of the body directly to the pulmonary arteries.

At the end of the treatment the right ventricle is better adjusted to work in a high-pressure system, but this inevitably leads to right ventricular failure at the age of ~30 and finally death [5]. Despite the improvements in

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surgical technique, it is a palliative, not a curative option. Cardiac transplantation in this group of patients is limited by the number of donors and has poorer results [6]. Therefore, a strong need to find some alternative treatment that could slow or ideally prevent the right ventricle from failing remains unaddressed.

Stem cell therapy may be an effective and safe option to ameliorate cardiac remodeling and improve single ventricle function. Preclinical studies using a swine right ventricle overload model have proven that ejection fraction in the stem cell treated group was significantly higher than in the placebo group [7]. Despite those favorable preliminary results, it has not been proven that stem cells can engraft and proliferate in failing myocardium [8]. The knowledge derived from the latest animal studies helped understand that stem cells actively participate in reducing inflammation and fibrosis while promoting restoration of cardiac function through their secretome [9]. Stem cell treated rat and porcine myocardium was characterized by high expression of antihypertrophic secreted factor, growth differentiation factor 15 (GDF-15) and SMAD2/3, which is believed to be GDF-15's downstream effector [7].

There are at least a few types of stem cells that are currently used in ongoing clinical trials.

They come from different tissues, from donors who differ in age, and finally from different environmental conditions. All these factors play an important role in cells' characteristics. Deep proteome analysis of neonatal cardiac progenitor cells (nCPCs) and adult cardiac progenitor cells (aCPCs) showed the significant difference in their secretome profile and different capabilities which derive directly from the paracrine activity [10].

The aim of this review is to present stem cell types used in experimental therapy, characterize the most important ongoing clinical trials, and explore potential future directions in translational medicine.

Stem cell types

Mesenchymal stem cells (bone marrow-derived)

They are derived from bone marrow stromal cells and can differentiate into bone, cartilage, ligament, tendon, muscle and adipose tissue [11]. They are characterized by expression of CD105, CD73, CD90, CD29, CD166 and the lack of CD45, CD34, CD14, CD11b, CD79α, CD19 and HLA-DR [11–13]. They have favorable characteristics for allogenic transplantation due to the lack of MHC II, CD80, BD86 and decreased MHC I. Allogeneic mesenchymal stem cell (MSC) transplantation through endocardium in a chronic pig model has been described to reduce infarct size and promote c-kit+ CSCs [14]. The safety of MSC transplantation was also demonstrated in a phase I double blind placebo controlled clinical trial in treatment of acute myocardial infarction [15]. The POSEIDON-pilot study and POSEIDON-DCM study were a continuation of transendocardial delivery of MSC [16, 17]. This second clinical trial demonstrated better ejection fraction in patients who received allogenic transplantation of MSC [17]. To date there has not been any trial using MSC for patients with single ventricle pathology.

Umbilical cord blood-derived cells

Mesenchymal stem cells isolated from umbilical cord blood can proliferate into bone, cartilage and fat cells as well as hepatocyte-like cells, neuroglial-like cells and endothelium of the respiratory tract and finally cardiomyocytes [18–20]. In animal studies this type of cells improved the myocardial function after myocardial infarction (MI) and in pressure overload models [21, 22]. The RIMECARD Trial has been the only one using umbilical cord blood (UCB)-derived MSC intravenously in adults with chronic heart failure and reduced ejection fraction (EF). The treatment was associated with the improvement of LVEF after 1 year and reduction in New York Heart Association (NYHA) class [23].

Cardiosphere-derived cells

Creation of cardiospheres is possible when myocardial biopsy samples are cultured *in vitro* on poly-D-lysine. They are multilineage and self-assembling. Cell clusters are composed of an outer layer of cardiac committed cells and an inner layer of multipotent stem cells [15, 24, 25].

Cardiosphere-derived cells (CDCs) respond to ischemia by promoting myocardial regeneration and increasing tissue resilience to insufficient blood supply [25]. CADUCEUS was the first clinical trial using autologous CDCs in 17 patients after MI which proved the safety of the therapy at 6 months. It also demonstrated smaller infarct scars, greater viable myocardial mass, and improved contractility and wall thickness in comparison to the control [26].

Cardiac progenitor cells

Cardiac progenitor cells (CPCs) are one of the best described in the literature. Surface receptor tyrosine kinase is highly expressed on their surface (C-kit+) unlike CD45, Lin or tryptase, which are absent in these cells. Preclinical animal models in both acute and chronic ischemia demonstrated the efficacy of CPCs in ameliorating LV dysfunction [27]. Dr. Kauhsal's group, in one of the largest and most detailed characterizations of CPCs, examined samples from the right atrial appendage (RAA) in young patients undergoing cardiac surgery procedures (due to different cardiac diseases) and observed that density of CPCs in the myocardium decreased with age of the patient. In neonates the density was 9% falling to 3% in older children [28, 29]. CPC density in the other part of the heart is extremely small [30]. It has been proved that not only age but different environmental conditions, such as hypoxia, may influence secretome production and paracrine capabilities of those cells. Sharma *et al*. noted

that neonatal CPCs had better regenerative potential in comparison to adult CPCs in an MI rat model. The LV EF was preserved at 7 and 28 days after injection [31, 32]. In histological examination of the infarcted samples, the rates of peri-infarct inflammation and fibrosis were significantly lower than in animals who received adult CPCs [31]. The other model which confirmed the unique abilities of neonatal CPCs in improving RV function was the pulmonary artery binding rat model [33]. The authors of that study isolated CPCs from age-varied human donors from neonates (0–1 month), and infants (1 month–1 year) to toddlers (1–5 years). The donor cells were given intraoperatively during the pulmonary artery binding procedure, directly to the myocardium. The 2-week follow-up revealed that rats treated with neonatal cells had improved RV function and tricuspid annular plane excursion (TAPSE) compared to controls. At 4 weeks after surgery the RV function remained unchanged. Animals which were given infant-derived CPCs showed no improvement after 2 weeks, but at 4 weeks TAPSE was higher than in placebo animals [33].

The first use of CPCs was the SCIPIO trial (stem cell infusions in patients with ischemic cardiomyopathy). During coronary artery bypass grafting (CABG) autologous cells were isolated from the right atrial appendage, expanded and administered intracoronarily (to the vessel supplying the infarct zone) 4 ±1 months after the initial surgery [34]. The investigators found that LVEF improved from 28% to 41% and the infarct size decreased by almost 40% [34]. At the same time a few papers have shown that CPCs lack the potential to differentiate into mature cardiomyocytes [35, 36]. That statement leads to a very important question: Is the secretome a real functional unit of the cells, and is the cells' paracrine capability what determines their efficacy? The paracrine activity of CPCs will be discussed in subsequent paragraphs of this review.

Stem cell therapy: preclinical models and paracrine activity

To understand the challenges of single ventricle physiology it is necessary to remember the differences between the RV and LV. In the normal adult heart, there is a muscular septum dividing the right and left ventricle from each other. When added to the interatrial septum it creates separate pulmonary and systemic circuits where each ventricle is anatomically and functionally adjusted to the demands of those circuits. The LV pumps against high resistance in the arterial vascular bed, which is why it has a thick wall and conical shape, inlet, and outlet on the same side. The LV works under high wall stress and is supplied from all 3 coronary arteries. The RV works against low resistance in the pulmonary vascular bed and is supported by the LV in diastole by creating negative pressure across the open mitral valve and provides suction. The negative pressure is transmitted to the pulmonary vessels and decreases RV afterload [37]. The RV's crescent shape and thin walls, obvious separation between inlet and outlet and blood supply by the single right coronary artery reflect the requirements of a low pressure system. The RV can adjust its work to different inflow conditions but is not able to work efficiently with high afterload. This remains the main challenge when the RV needs to work as a systemic chamber after surgical palliation in HLHS. Working in a high-pressure setting induces myocytes' hypertrophy and angiogenesis, which are positive at the beginning but end up with fibrosis and loss of contractility. The differences between the two chambers originate from different gene expression in the two heart fields from which the RV and LV are created. Those different origins of the ventricles and gene expression have been the subject of multiple studies.

In preclinical studies testing pressure overload, human MSCs and c-kit+ CPCs were evaluated in a juvenile swine model after pulmonary artery binding (PAB) [7, 38]. One million cells were administered intramyocardially into the RV free wall. In echo measurement the RV dilatation was reduced, and the RV systolic function was preserved in the treated versus control group. On the tissue level reduced fibrosis, increased angiogenesis, cardiomyocyte, and endothelial proliferation were found [38]. The mechanism of action is based on growth differentiation factor 15 (GDF-15), which belongs to the transforming growth factor β superfamily (TGF-β) which attenuates the hypertrophic response to the pressure overload [38]. Similar findings in an ovine PAB model were demonstrated with UCB-derived mononuclear cells [39]. Besides those promising results one problem remains unsolved – both the engraftment and differentiation of exogenous stem cells are very low [40]. There is growing evidence that rather than engraftment and differentiation the secretion of growth factors plays the key role in neovasculogenesis, favorable remodeling and activation of endogenous stem cells and cardiomyocytes, leading to overall improvement in cardiac function [41]. In a rat model of MI, the secretome was found to be directly correlated with the stem cell donor's age [10, 33]. The recovery was proven despite the very low amount of either cell type identified by polymerase chain reaction (PCR). Kaushal's group demonstrated that nCPC or a CPC-derived secretome was at least as effective as live cell transplantation in recovering from MI. The study group treated with nCPC-derived secretome maintained the improvement in ventricle function until the end of the study – at 28 days [10]. The same authors stated that the nCPC secretome acts through the heat shock pathway via differential expression of heat shock factor 1 (HSF1) [10]. This mechanism of action was confirmed *in vitro* by knocking down HSF-1 in nCPCs and overexpressing HSF-1 in aCPCs. Quantitative PCR revealed that HSF-1 knock-

down in nCPCs reduced expression of hypoxia-inducible factor-1α, VEGF, HSF-2, HSP 90AB, HSP70, and HSPD1 by 50%. Overexpression of HSF-1 in aCPCs caused a 2-to-3-fold increase in the levels of those proteins [10]. Modified nCPCs lost their resistance to oxidative stress, reduced metabolic activity, and did not proliferate effectively. At the same time aCPCs showed just the opposite capabilities [10].

In the above experiment the new characteristics of cells were reflected in the change of the secretome [10].

Clinical trials of stem cell therapy in children

According to http://clinicaltrials.gov there are more than 100 active clinical trials testing MSCs in the United States alone. In comparison there are not many trials including patients with congenital heart defects (CHD). Almost all of them target children with single ventricle pathology.

A summary of the clinical trials is presented in Table I.

TICAP, PERSUES and APOLLON demonstrated safety and efficacy of stem cell therapy. The investigators reported that younger age was related to a larger increase in ejection fraction – 10–15% at age 1 and approximately 5% at age 3 [42].

The ELPIS trial, which combined stem cell therapy with the surgical palliation, investigated the safety and feasibility of intramyocardial administration of allogeneic MSCs versus autologous preparation at the time of the second stage operation in HLHS [43]. The trial was ended and continued as longeveron mesenchymal stem cells (LMSCs) Delivered During Stage II Surgery for HLHS. Serious adverse events will also be monitored as well as cardiac function and somatic growth.

The mentioned trial investigated stem cell therapy at stage II or III of HLHS surgical treatment. One can speculate whether there would have been beneficial outcomes if the treatment had been applied earlier. On the other hand, the mortality between stage I and II is usually higher, which makes this time unfavorable as far as designing the trial is concerned. However, the early age population will have to be addressed as soon as this treatment is well introduced and established.

Are children the best possible recipients of stem cell therapy?

The results of stem cell therapy for ischemic heart disease in adults have been inconsistent to date [44]. Children may turn out to be more receptive to stem cell signals and their myocardium may be responsive to stem cell therapy according to several studies [28, 45–48]. Parmacek *et al.* demonstrated using carbon-14 dating that cardiomyocyte turnover is maximally 1% within a year just after birth and declines to 0.45% later in childhood [45]. Histone phosphorylation analysis proved that cardiomyocytes lose the majority of their cell cycle activity at the age of 20 years [46]. The density of cardiac cells in the myocardium decreases with age, as already mentioned [28]. All the above findings show that myocardium is plastic in the early stage of human development, which was confirmed by improvements after injection of MSCs, UCB-derived MSCs and CDCs in children [47, 48].

Route of administration

The most common technique of intracoronary administration is repeated occlusion of the target vessel with the angioplasty balloon. The cells are injected distally to the occlusion. The occlusion is maintained no longer than 2 min. This method was used both in TICAP and PERSEUS trials in children and proved to be safe and effective. The only challenge was assessing the coronary ostia in pediatric patients. There was a transient periprocedural increase of troponin, but no evidence of MI was reported. Some concerns about the possibility of coronary occlusion were brought to light. If the cells are very large and the coronary vasculature in children is very small, this risk may be real, especially as the stop-flow technique of MSC administration was associated with coronary occlusion in animal models [49–51]. Intramyocardial injection may be a safe alternative especially in children undergoing open heart procedures. To make it safe the total amount of the proper dose is divided into many small aliquots directly injected into a free wall of the RV. This way of delivery was validated in preclinical settings and is used in ongoing clinical trials in children (Table I).

New trends in stem cell therapy

Because the engraftment and retention of transplanted cells are rather poor, there is a strong need for alternative stem cell derived products. The secretome is now proved to be the functional unit of the stem cell. The cocktail of growth factors produced by the stem cells was successfully used for the treatment of injured myocardium. A single dose of total conditioned medium (TCM) derived from neonatal CPCs was more successful in improving cardiac function in a rat MI model in comparison to live transplanted nCPCs [10]. The same investigators isolated the exosomal fraction from TCM and injected it in the same model, which resulted in increased functional recovery in comparison to live cell injection [10]. The intravenous use of the secretome itself as a therapeutic agent is now being tested in a large animal model and the results are to be published later this year. It looks highly possible that the future of stem cell therapy will rely on customized secretome-derived products which do not need immunosuppression therapy, are safe, effective, easy to administer and ideally can be off-the-shelf products.

Conclusions

There have been several both clinical and preclinical studies to support the safety and efficacy of stem cell

Table I. List of stem cell clinical trials in single ventricle heart

Trial	Year	Design	Sponsor	Cell type	Route of administration	Time	Status
TICAP Transcoronary Infusion of CPCs in Patients with Single Ventricle Physiology NCT01273857	2011	Nonrandomized controlled phase I	Okayama University National Cerebral and Cardiovascular Center, Japan	CPC autologous	Intracoronary	Stage II or III operation	Completed
PERSEUS Cardiac Progenitor Cell Infusion to Treat Univentricular Heart Disease NCT01829	2013	Randomized controlled phase II	Okayama University Translational Research Informatics Center, Kobe	CPC autologous	Intracoronary	Stage II or III operation	Completed
Safety Study of Autologous Umbilical Cord Blood Cells for Treatment of HLHS NCT01883076	2013	Single treatment group phase I	Mayo Clinic University of Oklahoma	UCB-derived MSCs autologous	Intramyocardial	Stage II operation	Active, non-recruiting
APOLLON Cardiac Stem/Progenitor Cell Infusion in Univentricular Physiology NCT02781922	2016	Randomized phase III	Kanagawa Children's Medical Center, Yokohama, Japan Okayama University, Okayama, Japan	Autologous cardiac stem cells (JRM-001)	Intracoronary	Stage II or III operation	Unknown
Mesoblast Stem Cell Therapy for Patients with Single Ventricle and Borderline Left Ventricle NCT03779711	2017	Randomized controlled phase I/II	Boston Children's Hospital, Boston, Massachusetts	MPCs (mesenchymal precursor cells) allogenic	Intramyocardial	Stage II or LV recruitment operation	Recruiting
Safety of Autologous Cord Blood Cells in HLHS patients During Norwood Heart Surgery NCT03431480	2018	Single group assignment phase I	Royal Children's Hospital, Melbourne, Victoria, Australia	Autologous human placental blood Mononuclear cells	Intracoronary	Stage I	Recruiting
Longeveron Mesenchymal Stem Cells (LMSCs) Delivered during Stage I Surgery for HLHS (ELPIS) NCT03525418	2018	Randomized controlled phase I/II	Emory University/Children's healthcare of Atlanta University of Maryland Medical Center John Hopkins University University of Utah	Longeveron mesenchymal stem cells (allogenic bone marrow-derived mesenchymal stem cells)	Intramyocardial	Stage II	Active, non-recruiting
Intramyocardial Injection of Autologous Umbilical Cord Blood Derived Mononuclear Cells During Surgical Repair of HLHS NCT03779711	2018	Nonrandomized controlled phase II	Children's of Alabama Children's Hospital of Los Angeles Children's Hospital Colorado Ochsner Medial Center Mayo Clinic	Autologous umbilical cord blood derived mononuclear cells	Intramyocardial	Stage II	Recruiting
CHILD The Child Trial: Hypoplastic Left Heart Syndrome Study NCT03406884	2019	Randomized controlled phase I/II	Emory University Children's Healthcare of Atlanta University of Maryland	c-kit+ cells	Intramyocardial	Stage II	Recruiting

therapy in children with single ventricle physiology. Preclinical studies involving swine and rodent models have proven the potential of stem cells to improve the cardiac function of ischemic and hypertrophic myocardium. The secretome has been identified as a functional unit of stem cell therapy. Further investigation needs to be performed to assess the optimal dosing, regimen, and route of delivery of stem cells as a therapeutic agent. As the stem cell therapy in children continues to evolve, investigators hope that this therapy will provide an effective way of treatment in congenital heart diseases, offering both longer life and better quality of life.

Conflict of interest

The authors declare no conflict of interest.

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