Right ventricular failure secondary to chronic overload in congenital heart diseases: Benefits of cell therapy using human embryonic stem cell–derived cardiac progenitors

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Objective: Despite the increasing incidence of right ventricular (RV) failure in adult patients with congenital heart disease, current therapeutic options are still limited. By contrast to left-heart diseases, cell-based myocardial regeneration applied to the right ventricle is poorly studied, even though it may be a therapeutic solution. As human embryonic stem cell–derived cardiac progenitors seem to be good candidates owing to their proliferation capacity, our aim was to assess, in a large animal model of overloaded RV dysfunction, the feasibility and effects of such a cell therapy.

Methods: Human MesP1$^+$/SSEA-1$^+$ cardiogenic mesodermal cells were administered using multiple intramyocardial injections 4 months after a surgical procedure mimicking the repaired tetralogy of Fallot, and their effects were observed 3 months later on hemodynamic, rhythmic, and histologic parameters.

Results: All pigs (sham n = 6, treated n = 6) survived without complication, and cell therapy was clinically well tolerated. Although functional, contractility, and energetics parameters evolved similarly in both groups, benefits regarding arrhythmic susceptibility were observed in the treated group, associated with a significant decrease of peri-myocyte fibrosis (5.71% ± 2.49% vs 12.12% ± 1.85%; $P < .01$) without interstitial fibrosis change (5.18% ± 0.81% vs 5.49% ± 1.01%). Such a decrease could be related to paracrine effects, as no human cells could be detected within the myocardium.

Conclusions: Cell therapy using intramyocardial injections of human MesP1$^+$/SSEA-1$^+$ cardiogenic mesodermal cells seems to have benefits regarding overloaded RV tissue remodeling and arrhythmic susceptibility, but this mode of administration is not sufficient to obtain a significant improvement in RV function. (J Thorac Cardiovasc Surg 2015;149:708-15)

See related commentary on pages 715-7.

Supplemental material is available online.

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Right ventricular (RV) failure remains a major problem in the long-term follow-up of patients with congenital heart disease, leading to impairment of functional status, severe arrhythmias, and premature death.[1] This RV dysfunction occurs in young adults as a consequence of chronic pressure and volume overload secondary to procedures that had early success, in patients with either 2 ventricles in which the right ventricle was abnormal (eg, tetralogy of Fallot [TOF]), or was the dominant or systemic ventricle (eg, hypoplastic left heart syndrome).[2] In these patients, more than 50% have a risk of heart failure after age 30 years[1] Moreover, RV function is a major prognostic factor of pulmonary artery hypertension, regardless of etiology.[3] Consequently, management of RV failure was identified as a priority in cardiology research.[4]

By contrast to left ventricular failure, clinical management of patients with RV failure remains challenging: drugs or resynchronization give variable benefits,[5] and surgical procedures such as valve replacement or repair may delay RV failure, but may fail to improve altered RV function. At the end-stage of heart failure, cardiac transplantation is mandated, but its application is restricted by the availability

Abbreviations and Acronyms
DNA = desoxuribose nucleic acid
ECG = electrocardiogram
ESC = embryonic stem cell
HD = high-density
PVS = programmed ventricular stimulation
RV = right ventricular
TOF = tetralogy of Fallot

of donor organs. Cell-based myocardial repair may be an alternative approach. Human embryonic stem cell (ESC) engraftments were successfully attempted in left ventricular myocardium after ischemic injuries.[6] Regarding the right ventricle, cell therapy using myoblastic[7] or cord-blood stem cells[8,9] has been attempted but yielded poor results.

Treating RV failure, in contrast to ischemic left ventricle, by cell therapy, has to take into account not only the postoperative scarred area, but also the specific geometry and physiologic processes of the right ventricle. Indeed, RV chronic overload alters the entire RV myocardium.[10,11] This extensive alteration requires a substantial number of cells to treat, and potentially induce a functional improvement of, the RV myocardium. Among multiple cell types considered as potential sources of cardiac progenitors, some did establish a functional coupling with the host myocardium.[6,12,13] ESCs derived from the inner mass of the blastocyst possess this capacity and are able to proliferate, differentiate in vivo into mature cardiac myocytes, and repopulate significant regions of the damaged myocardium.

A proof of concept has been reported in a nonhuman-primate model of infarcted myocardium.[6,13] In one of these models,[13] the maturation and differentiation of cardiac progenitors into ventricular myocytes seemed to be optimal in areas composed of both fibrosis and damaged cardiac fibers. Instances in which such a structural remodeling constitutes a favorable environment for stem-cell grafting has been observed in patients with overloaded RV dysfunction,[14] suggesting that cardiac progenitor cell therapy may be applied in this indication.

The purpose of our study was to evaluate the feasibility and effects of a cell therapy using human ESC-derived mesodermal cardiogenic cells in our porcine model of chronic overloaded RV dysfunction.[10] The impact of this treatment on hemodynamic and rhythmic parameters was evaluated throughout the follow-up; the fate of cardiac progenitors and the RV structural remodeling were assessed.

METHODS

Experimental Design

Twelve Landrace male piglets were studied in accordance with European Union regulations (Directive 86/609 EEC). This study was approved by the French Ministry of Agriculture (approval No. B92-019-01) and the Committee on Ethics of Animal Experiments CEEA26 CAPSud. Animals underwent an electrocardiogram (ECG) and hemodynamic evaluation at 3 points in the experiment: baseline, and 4 and 7 months of follow-up. The surgical procedure mimicking repaired TOF was performed after the baseline step. Briefly, an enlargement of the RV outflow tract by a polytetrafluoroethylene patch, excision of 1 pulmonary valve leaflet, and a pulmonary artery banding were performed.[10] The rhythmic risk was evaluated throughout the cell-therapy period. Histologic analyses were performed on each animal at the end of the procedure.

Cardiac Progenitor Characteristics and Implantation into the RV Myocardium

Human MesP1$^+$ (mesoderm posterior 1)/SSEA-1$^+$ (stage-specific embryonic antigen-1; CD15) cardiac progenitor cells derived from the HUES-24 (human embryonic stem) cell line were used. Briefly, HUES-24 cells were treated with 100-ng/ml Wnt3a for 24 hours and then with 10-ng/ml BMP2 for 3 days; cells were sorted with a biotin anti-CD15 antibody (Exbio, CliniSciences, Nanterre, France) and antibiotin conjugated beads (Miltenyi Biotec, Paris, France). RNAs extracted from both CD15$^+$ and CD15$^-$ cells were submitted to reverse transcriptase. Complementary DNAs were run in a real-time polymerase chain reaction, as described elsewhere.[13] Cell characteristics are presented in Figure 1. The selection excludes undifferentiated or early neural stem cells, and no teratoma or proliferative cell foci formation was detected on explanted heart, lung, or liver using scanner multislices imaging (Somatom Definition Flash, Siemens Healthcare, St. Denis, France).

Cell transplantation was performed 4 months after surgery through a right thoracotomy approach. Animals received either medium containing mesodermal cardiogenic cells (treated group: n = 6) or vehicle alone (sham group: n = 6). The total bolus (10^7 cells) was injected into the RV free wall at 20 separate injection sites; 20 other injections were made in a high-density (HD) area of 1 cm^2 identified by nonabsorbable sutures, using a 28-gauge needle (3 mm deep, 25 μl/site) connected to a mesotherapy pistol (DHN-2, Techdent, Sallanches, France). Hydrocortisone (1 mg/kg) was injected intravenously before closing, to reduce inflammation. All animals were immunosuppressed by tacrolimus by mouth (0.3 mg/kg/day, plasmatic level: see Table 1).

ECG and Rhythm Study

A 12-lead surface ECG was recorded, and QRS duration was analyzed.[10] An insertable recorder (Reveal, Medtronic France SAS, Boulogne-Billancourt, France) was implanted subcutaneously under the left scapula at the time of cell implantation, to record the heart's rhythm until the end of follow-up. This cardiac monitor was programmed to record tachycardia >200/minutes, during at least 6 complexes. As a last step, a stored ECG was collected by percutaneous interrogation, and a programmed ventricular stimulation (PVS) was carried out with a quadripolar catheter inserted into the RV apex through the femoral vein.

Standard clinical PVS protocols were employed, including application of single, double, and triple extrastimuli of increasing prematurity until reaching the RV refractory period, after a sequence of 8 conditioning stimuli. The heart was then challenged 3 times with a sequence of 8, followed by a single extrastimulus at compulsory rhythms of 100, 120, and 150/minutes. If no ventricular tachycardia was induced, this procedure was repeated to apply 3 challenges with double, and if necessary triple, extrastimuli. The PVS was considered positive if these challenges produced sustained ventricular tachycardia (>30s) or ventricular fibrillation.

Hemodynamic Study

Quantification of RV overload and contractile performance of RV myocardium were assessed by the conductance catheter technique. Briefly, the conductance catheter was inserted in the right ventricle through the