Disturbed myocardial connexin 43 and N-cadherin expressions in hypoplastic left heart syndrome and borderline left ventricle

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Objectives: Borderline left ventricle is the left ventricular morphology at the favorable end of the hypoplastic left heart syndrome. In contrast to the severe end, it is suitable for biventricular repair. Wondering whether it is possible to identify cases suitable for biventricular repair from a developmental viewpoint, we investigated the myocardial histology of borderline and severely hypoplastic left ventricles.

Methods: Postmortem specimens of neonatal, unoperated human hearts with severe hypoplastic left heart syndrome and borderline left ventricle were compared with normal specimens and hearts from patients with transposition of the great arteries. After tissue sampling of the lateral walls of both ventricles, immunohistochemical and immunofluorescence stainings against cardiac troponin I, N-cadherin, and connexin 43, important for proper cardiac differentiation, were done.

Results: All severely hypoplastic left hearts (7/7) and most borderline left ventricle hearts (4/6) showed reduced sarcomeric expressions of troponin I in left and right ventricles. N-cadherin and connexin 43 expressions were reduced in intercalated disks. The remaining borderline left ventricle hearts (2/6) were histologically closer to control hearts.

Conclusions: Four of 6 borderline left ventricle hearts showed myocardial histopathology similar to the severely hypoplastic left hearts. The remainder were similar to normal hearts. Our results and knowledge regarding the role of epicardial-derived cells in myocardial differentiation lead us to postulate that an abnormal epicardial–myocardial interaction could explain the observed histopathology. Defining the histopathologic severity with preoperative myocardial biopsy samples of hearts with borderline left ventricle might provide a diagnostic tool for preoperative decision making. (J Thorac Cardiovasc Surg 2012;144:1315-22)

Hypoplastic left heart syndrome (HLHS) is a spectrum of morphologic abnormalities of the left heart first described by Noonan and Nadas.[1] At the severe end, the left ventricle is vestigial, in combination with aortic and mitral atresia. In cases with mitral stenosis, the hypoplastic left ventricle can show endocardial fibroelastosis with aortic atresia or severe aortic stenosis.[2] In contrast to the original definition, the term *HLHS* has often been used to refer only to the severe end of this spectrum. In this report, we use the term *HLHS* as originally described. The term *hypoplastic left heart complex* was introduced to refer to a subset at the mild end of the HLHS spectrum, comprising small but functional left ventricles without endocardial fibroelastosis and no valvular atresia or severe stenosis.[3] The term *borderline left ventricle (BLV)* has been used to indicate this favorable end of the HLHS spectrum.[4] In this study we use the term *hypoplastic left heart (HLH)* to indicate the phenotype of the left ventricle at the severe end of the HLHS spectrum and *BLV* to indicate the phenotype of the left ventricle at the favorable end.

Different therapeutic strategies have been described that can be applied to neonates with HLHS.[5] Cardiac transplantation and univentricular surgical correction are the 2 possibilities for the HLH phenotype. For the BLV phenotype, both univentricular and biventricular repairs have been described.[5,6] Determination of whether a left ventricle is adequate to maintain the systemic circulation is, however, a major challenge in the decision making before the operative correction. Despite reports in the literature of the use of multiple diagnostic tools to aid in this decision making, a conclusive diagnostic tool is still missing.[6-8] A possible solution could be found in a different developmental background of the myocardium of the hearts with BLV and HLH phenotypes. In particular, very little is known about the histology of left ventricular myocardium across the HLHS spectrum.[2,9-11]

Recent studies on cardiogenesis have provided new insight into the origin of the ventricular myocardium,

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Abbreviations and Acronyms

BLV = borderline left ventricle
Cx43 = connexin 43
EPDC = epicardium-derived cell
HLH = hypoplastic left heart
HLHS = hypoplastic left heart syndrome
ICD = intercalated disk
PBS = phosphate-buffered saline solution

enabling a better approach to understanding the developmental background of the myocardium in HLHS. The myocardium of the left and right ventricles is derived from different progenitor cell populations, the first and the second heart fields, respectively.[12] The epicardium originates from the second heart field and gives rise to epicardium-derived cells (EPDCs). These EPDCs migrate into the myocardial wall and, through epicardial–myocardial interactions, exert an essential role in establishing myocardial architecture.[13,14]

Studies in the past 2 decades showed that electromechanical coupling between the cardiomyocytes is essential for myocardial differentiation.[14-16] In particular, the intercalated disk (ICD) has a major role in the regulation of the electromechanical transduction, incorporating adherence junctions, desmosomes, and gap junctions. Adherence junctions and desmosomes provide N-cadherin–mediated intercellular adhesion and function as a platform for cytoskeletal anchorage, whereas gap junctions composed of connexin 43 (Cx43) are involved in cell–cell exchange.[15,16] Recently, we investigated the role of EPDCs in myocardial maturation in the mouse[14] by studying the expression patterns of troponin I, N-cadherin, and Cx43. Several EPDC-depletion models during development show myocardial abnormalities, including ventricular noncompaction and downregulation of myocardial N-cadherin and Cx43 expressions.[14]

In this study, we investigated normal human postmortem hearts and compared these with specimens with HLH and BLV phenotypes by using the aforementioned markers involved in myocardial differentiation and ICD function. Additionally, we evaluated the staining of these markers in hearts from patients with transposition of the great arteries to ensure that the observations made in HLH and BLV were specific to these malformations. We hypothesized that BLV and HLH might present with myocardial histopathologic abnormalities in varying degrees of severity, which might be caused by abnormal myocardial development and maturation regulated by EPDCs. Moreover, we hypothesized that our findings might be helpful in providing an additional diagnostic tool for surgical decision making.

MATERIALS AND METHODS

Selection

Twenty-two human heart specimens from patients who had not undergone operation, comprising 6 with normal anatomy, 7 with HLH phenotype, 6 with BLV phenotype, and 3 with transposition of the great arteries, were studied. The hearts were selected according to the definition criteria in the literature, as explained previously. In the HLH group, hearts with mitral and aortic valve atresia as well as hearts showing mitral valve stenosis and aortic valve atresia were included. Hearts with BLV phenotype were defined as those in which the apex was formed by the right ventricle and the left ventricular lumen was at least a third of the base–apex distance of the right ventricle in the absence of right-sided abnormalities. The left ventricle in BLV was small but functional, without macroscopic myocardial abnormalities, valvular atresia, or severe stenosis. In the normal hearts, the left ventricle was larger than the right ventricle and the apex was formed by the left ventricle without other morphologic abnormalities.

Morphologic measurements of cardiac structures were performed to refine the selection. The length of the left ventricular lumen was measured from the mid aortomitral continuity to the compact myocardium at the apex, whereas the right ventricular lumen was measured from the base of the pulmonary valve to the compact myocardium at the apex. A *Z* score for the morphologic measurements was calculated (Table 1).

The hearts were obtained from the Leiden Collection of malformed hearts (Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, The Netherlands). This collection includes hearts preserved in ethanol and glycerin, dating from the early 1950s to the recent era. This study was undertaken in accordance with the Dutch regulation for the proper use of human tissue for medical research purposes.

Sampling

Transmural tissue blocks of approximately 5× 5 mm were taken from the free walls of both ventricles. The site of sampling was standardized at the proximal third of ventricular base–apex length. The embedding was standardized, and the samples were collected in 70% ethanol. The samples were routinely processed for paraffin embedding and immunohistochemical investigation. Transverse sections (5 μm) in the craniocaudal direction were mounted on albumin and glycerin–coated glass slides in order from 1 through 10.

Immunohistochemical Examination

After deparaffinization and rehydration of the slides, microwave antigen retrieval was applied by heating 12 minutes at 98°C in a citric acid buffer (0.01 mol/L in distilled water, pH 6.0). Inhibition of endogenous peroxidase was performed for 20 minutes with a solution of 0.3% hydrogen peroxide in phosphate-buffered saline solution (PBS). The slides were incubated overnight with the primary antibody anti–troponin I (1/200), dissolved in PBS and polysorbate 20 with 1% bovine serum albumin. Between subsequent incubation steps, all slides were rinsed in PBS (2 times) and PBS and polysorbate 20 (1 time). The slides were incubated 60 minutes with the secondary antibody goat anti–rabbit biotin (1/200) in goat serum (1/66) in PBS and polysorbate 20. Subsequently, all slides were incubated 45 minutes with avidin–biotin complex reagent. For visualization, the slides were incubated 10 minutes with 400 μg/mL 3-3′diaminobenzidine tetrahydrochloride dissolved in tris(hydroxymethyl)aminomethane maleate buffer (pH, 7.6) to which 20 μL hydrogen peroxide was added. Counterstaining was performed with 0.1% hematoxylin for 5 seconds, followed by rinsing 10 minutes with tap water. All slides were dehydrated, mounted with Entellan (Merck & Co, Inc, Whitehouse Station, NJ), and studied under light microscopy.

Immunofluorescence

Double stainings for N-cadherin (1/500 mouse monoclonal antibody GC-4; Sigma-Aldrich Co LLC, St Louis, Mo) and Cx43 (1/200 rabbit