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Original Article

Integrin expression during reverse remodeling in the myocardium of heart failure patients

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Abstract

Background: The main anchoring proteins of myocardial cells with each other and with the extracellular matrix are integrins present in the membranes of myocardial cells. These integrins are important for maintaining the architecture of the myocardial tissue and the mechanotransduction in the heart. Heart failure leads to various alterations in the myocardium, such as changes in morphology, and in expression of mRNAs, miRNAs, and proteins. Left ventricular assist device (LVAD) support in heart failure patients has been described to induce reverse remodeling of the myocardium and thus to (some degree of) reversal of the aforementioned alterations. In this study, we evaluated whether changes in expression of integrins α-1, -3, -5, -6, -7, -10, -11 and β-1, -3, -5 and -6 play a role during reverse remodeling. **Methods:** Three-step immunoperoxidase staining procedures were applied on frozen heart tissue sections to locate the various integrins tested. Integrin mRNA expression was established by standard Q-PCR procedures. **Results:** It was shown that mRNA expression of several integrins changes significantly during LVAD support, however without subsequent changes in immunohistochemical detectable quantities. Various integrins showed different locations within the myocardium. **Conclusion:** LVAD-induced reversed remodeling did not result in significant integrin protein expression, although changes in integrin mRNA expression suggested an adaptation to unloading.

Keywords: Integrins; Heart failure; Remodeling; LVAD support

1. Introduction

Trans membrane receptors such as integrins are important for the dynamic interaction between intracellular processes and the extracellular environment [1,2]. Integrins are expressed in all cellular compartments of the myocardium. They are critical to its form and function and are essential in regulating cellular processes [1–3]. Anchoring cardiomyocytes to the extracellular matrix (ECM) is mainly mediated by integrins and in this respect very important for maintaining the proper architecture of the total myocardium and for the mechanotransduction [4]. Structural remodeling during the development of heart failure is characterized by rearrangement of the architecture of the cardiac ventricular wall. It involves among others hypertrophy of the myocytes, fibroblast proliferation, increased deposition of ECM proteins, and altered expression of miRNAs [5–7].

Left ventricular assist devices (LVAD) are mostly used as bridge to heart transplantation (HTx) in patients suffering from end-stage heart failure and induces partial recovery of ventricular functions [8], improved condition of the patients [9], reduction in cardiomyocyte size [10], changes in contractile fibers [11,12], and depending on the type of heart failure [ischemic heart disease (IHD) or dilated cardiomyopathy (DCM)], to partial recovery of miRNA

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expression [7]. Furthermore, structural and volume changes of ECM and basal membrane components have been described [13].

As both cardiomyocyte size and ECM volume changes during LVAD support, we wondered how integrins as anchoring proteins between both alter during this support. The goal of this study was to analyze the changes in mRNA expression by quantitative PCR of several integrins (α1, -3, -5, -6, 7,- 10, -11 and β-1, -3, -5 and -6) in the myocardium of heart failure patients before and after LVAD support. To establish the location of integrin-α5, -α6, -α7, -β1 and β6, immunohistochemical techniques have been used. Previously, we showed that collagen IV expression diminished in the basal membrane after LVAD support. This is in contrast to laminin that did not alter [13]. To explore the role of the basal membrane further, also the changes in perlecan expression were studied. Perlecan is an important heperan sulfate proteoglycan in the basal membrane; its functions in anchoring matrix proteins and its expression change with mechanical stretch [14].

2. Methods

2.1. Patients and tissue samples

Sixteen patients (age: 38±12 years; 14 men and 2 women) with refractory end-stage heart failure diagnosed with IHD (n=7) or with DCM (n=9) were selected for this study (Table 1). Because of the different etiologies of DCM and IHD, both groups were analyzed separately. All patients were treated with a pneumatic LVAD (Heart-Mate I, Thoratec, Pleasanton, CA, USA) as a bridge to HTx, between 2000 and 2005. At the time of LVAD implantation, all patients were in NYHA functional class IV, and in NYHA functional class I while on LVAD support. Prior to LVAD implantation, all patients received intravenous inotropics because of hemodynamic deterioration. Cardiac medication was discontinued initially in all patients after LVAD implantation (except for aspirin), but resumed if necessary (Table 1). Informed consent to participate in this study was obtained from all patients before LVAD implantation. The pre-LVAD biopsy (LV apical core) was obtained at the time of LVAD implantation. These biopsies were compared with LV tissue specimens of the explanted heart after HTx (post-LVAD), taken from the apical half of the LV. All biopsies were directly frozen. Normal myocardial tissue was obtained from vital organ donors from which the heart could not be used because of noncardiac reasons (n=2) and from autopsy on patients with no pathology of the heart (n=3). These biopsies served as a control.

2.2. Immunohistochemistry

For the immunohistochemistry (IHC) of integrins, only (monoclonal) antibodies were selected that showed a strong staining without aspecific background on myocardial tissues. Therefore, only a limited number of integrins could be tested by IHC. Three-step immunoperoxidase staining to detect the localization of various integrins (and perlecan) was performed on sections prepared from frozen heart tissue samples obtained pre- and post-LVAD. Eight-micrometer-thick sections were mounted on silan-coated glass slides. Frozen sections were air dried at room temperature, fixed in acetone (10 min), washed in PBS/Tween-20 for 10 min, and incubated with the primary antibodies (goat anti-integrin α-5; -anti-integrin α-6, and -anti-integrin α-7, mouse anti-integrin β-1D or rabbit anti-integrin β-6; Table 2) for 1 h at room temperature.

Next, sections were washed in PBS/Tween-20 (10 min) and fixed in formalin (4%) to cross link the antibody to the tissue. Endogenous peroxidase was blocked by incubation in a blocking buffer (20 min) followed by washing in PBS/Tween-20 (30 min), and the sections were incubated

Table 1
Patient characteristics

No.	Age	Diagnosis	Gender	Days on LVAD	Medication during LVAD support
1	56	IHD	Male	138	None
2	57	IHD	Male	225	None
3	45	IHD	Male	259	2.5 mg Ramipril
4	57	IHD	Male	263	None
5	36	IHD	Male	325	2×4 mg Perindopril
6	26	IHD	Male	357	None
7	39	IHD	Male	548	3×6.25 mg Capoten
8	34	DCM	Female	55	3×6.25 mg Capoten
9	17	DCM	Male	111	None
10	47	DCM	Male	190	None
11	35	DCM	Male	196	None
12	32	DCM	Female	219	3 mg Captopril
13	25	DCM	Male	263	1×25 mg Losartan
14	32	DCM	Male	286	4 mg Perindopril
15	25	DCM	Male	330	2×10 mg Fosinopril
16	46	DCM	Male	484	3×50 mg Capoten

DCM: dilated cardiomyopathy, IHD: ischemic heart disease, LVAD: left ventricular assist device.