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Original Article

Cardiac molecular markers of programmed cell death are activated in end-stage heart failure patients supported by left ventricular assist device

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ABSTRACT

Background: Cardiomyocyte apoptosis increases in heart failure (HF) and is implicated in disease progression. The apoptotic cell is not inevitably committed to death, and appropriate therapy like left ventricular assist device (LVAD) support could offer a rescue of cellular functions. Literature data regarding the modulation of the apoptotic process during LVAD support are still controversial.
Methods: To assess whether LVAD implantation modifies the apoptotic profile in the heart, cardiac tissue was collected from end-stage HF patients before LVAD implant (pre-LVAD, $n=22$) and at LVAD removal (post-LVAD, $n=6$) and from stable HF patients on medical therapy without prior circulatory support (HTx, $n=7$) at heart transplantation as control. Caspase (Casp)-3, Bax, Bcl-2, and Hsp72 cardiac mRNA and protein expression were evaluated by real-time polymerase chain reaction and Western blotting (WB) in the three groups of patients. Immunohistochemical analysis, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay, and DNA laddering analysis were performed; cellular size and interstitial fibrosis content were also determined.
Results: All the apoptotic indices were increased in the post-LVAD group compared to pre-LVAD, specially antiapoptotic Hsp72 and proapoptotic Bax (Hsp72: 3.27 ± 0.41 vs. 0.76 ± 0.14, $P<.001$; Bax: 2.15 ± 0.38 vs. 1.10 ± 0.29, $P=.035$; post-LVAD vs. pre-LVAD, respectively). The significant increase in Hsp72 was confirmed by WB and immunohistochemical analysis.
Conclusion: LVAD appears to induce an activation of apoptotic mediators, mainly at the mitochondrial level, while the following activation of Casp-3 is reduced by the significant increase of Hsp72, whose enhancement could be an important factor in cardiac remodeling associated with LVAD support.

1. Introduction

Implantation of a left ventricular assist device (LVAD) is an established treatment able to improve survival and quality of life for patients with end-stage heart failure (HF) [1]. The purpose of LVAD support is to unload the failing heart and help restore cardiac function, improving cardiac output and organ perfusion. In HF patients, overload-induced distension of the heart causes a number of structural, functional, and molecular alterations known as cardiac remodeling, which in turn promotes HF progression [2–4]. Mechanical distension of the myocardium induces apoptotic death of cardiomyocytes [5], and apoptosis has been found in ventricles of subjects with end-stage HF undergoing cardiac transplantation [6,7]. The resulting loss of cardiomyocytes contributes to HF progression [8,9]; one of the main goals for reducing mortality and morbidity associated with HF is to preserve the number of existing myocytes, modulating the cell death process [10]. At the cellular level, HF is associated with a down-regulation of antiapoptotic biomarkers such as Bcl-2 [11,12], an important molecule for cell survival protecting cardiac myocytes against cell death by inhibiting activation of proapoptotic Bax [13].

There is evidence that LVAD positively interferes with cardiac remodeling, inducing the so-called "reverse remodeling" [14]. After LVAD, the hemodynamic improvement that allows weaning from the

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device has been observed in 5%–10% of patients [15], suggesting that LV unloading by LVAD can reverse many biochemical processes activated during HF progression. Increased expression of proinflammatory cytokines has been described in patients with chronic HF [16,17]. Inflammation has been implicated in tissue repair and/or wound healing in many organs, and the role of inflammation and potential impact of modulating inflammatory pathways in cardiac repair and regeneration is still controversial [18]. Interelukin (IL)-6 has a controversial role due to its antiapoptotic effects; tumor necrosis factor (TNF)-α plays an important role in cardiac remodeling by inducing apoptosis [8,19], although TNF-α stimulates nitric oxide production [20,21], which potentially induces the expression of several cytoprotective genes such as Hsp72, which in turn protects cells from apoptosis [22].

In failing human hearts with mechanical unloading, autophagy markers and mediators are decreased at the mRNA and protein level [23]. Although the signals regulating pathways of autophagy and apoptosis in the heart are closely linked [24,25], few data are available on the possible effect of LVAD implantation on the cardiac apoptotic process, although it has been hypothesized that cardiomyocyte apoptosis could be positively modulated by therapeutic treatment [26,27]. A markedly high gene expression of Fas, a transmembrane receptor of the TNF family able to promote apoptosis, has been observed at the time of LVAD support, whose effect was highly variable among individuals [28].

The aim of this study was to evaluate whether LVAD is able to inhibit the apoptotic process in the hearts of end-stage HF patients by measuring the expression of molecules involved in the activation of the apoptotic process, such as Bax, a hallmark of mitochondrial dysfunction; Caspase (Casp)-3, the effector of apoptosis; and the apoptosis inhibitors Bcl-2 and Hsp72.

The specific aims were:

1. to evaluate the apoptotic profile in HF patients undergoing LVAD implantation (pre-LVAD group), using a group of stable HF patients who were successfully transplanted without previous LVAD support (HTx group) as control.
2. to evaluate the effect of LVAD support on apoptosis, comparing the pre-LVAD group with patients at the time of LVAD removal (post-LVAD group).

2. Methods

2.1. Patients

End-stage HF patients (n=22) who underwent LVAD implantation as a bridge to heart transplantation were enrolled in the study (pre-LVAD group). All patients were supported by axial continuous-flow devices; 16 were HeartMateII LVADs (Thoratec, Pleasanton, CA, USA), 4 were Incor LVADs (Berlin Heart AG), 1 was a De Bakey LVAD (MicroMed Technology, Inc., Houston, TX, USA), and 1 was a HeartWare LVAD (HeartWare International Inc., Framingham, MA, USA).

In order to evaluate the apoptotic process in end-stage HF patients and the effect of LVAD support, apoptotic and antiapoptotic mediators determined in the pre-LVAD group of patients were compared with another two different groups of patients, respectively: (1) HTx group (control): seven stable chronic HF patients who underwent elective heart transplantation on medical therapy, without prior circulatory support, (2) post-LVAD group: five patients were supported by LVAD implanted as a bridge-to-heart transplantation and supported by axial continuous-flow devices [three were De Bakey LVADs (MicroMed Technology), two were HeartMateII LVADs (Thoratec)], while one patient was supported by a pulsatile-flow device (NewCorTec, Rome, Italy).

Regarding the pre-LVAD and the HTx patients, median age was comparable and idiopathic dilatative cardiomyopathy (IDC) was prevalent in both groups. In post-LVAD patients, the median support time prior to heart transplantation was 367 (152–483) days. All LVAD recipients experienced postoperative hemodynamic improvement with respect to condition at preimplant (data not shown). At heart transplantation, the levels of cardiac index and pulmonary capillary wedge pressure were comparable to those of HTx patients. No histological evidence of active myocarditis or inflammatory reaction was found in LVAD patients.

2.1.1. Inclusion and exclusion criteria for patients enrollment

Enrollment criteria for the LVAD implant were: idiopathic dilated/ischemic cardiomyopathy, not amenable to recovery by pharmacological or conventional surgical therapy; INTERMACS profile 1, 2, and 3 [29,30]; LV ejection fraction (LVEF) <25%; peak oxygen consumption <12 ml/kg/min; body surface area >1.5 m^2; urgent heart transplantation not feasible; lack of contraindications for LVAD implantation; acceptable overall operative risk. Exclusion criteria were: irreversible renal/hepatic failure due to preexisting chronic hepatorenal disease, severe diabetes mellitus with end-organ damage, severe peripheral vascular disease, coexisting active neoplasm, pregnancy, recurrent alcohol and drug abuse, and cognitive impairment severe enough to limit comprehension. As to the HTx group, end-stage HF patients, matched for age, sex, diagnosis, and New York Heart Association classes with LVAD candidates, were enrolled as control group.

2.2. Cardiac samples

Cardiac samples were taken from five different areas of the LV and right ventricular (RV) walls at the time of heart transplantation in both post-LVAD and HTx patients as previously reported [31]; samples from LV apex were taken from near the distal left anterior descending (LAD) coronary artery. LV anterior basal and the lateral basal samples were collected from near the proximal LAD (to the left of the pulmonary trunk) and to the first obtuse marginal branch (close to the left auricula), respectively; RV samples were obtained 1 cm from the right atrioventricular groove, below the origin of the pulmonary trunk and from the acute margin of the heart.

In the pre-LVAD group, tissues were collected at the time of LVAD implantation from the portion of LV apex excised during standard surgical procedure, whereas in post-LVAD patients, apical samples were taken at least 2 cm away from the inflow cannula to rule out myocardial tissue sample bordering the area affected by the sewing ring of the apex.

Macroscopic signs of fibrosis were avoided in the sample collection, resulting in n=30 myocardial samples in the post-LVAD group, n=35 in the HTx group, and n=22 in the pre-LVAD group. Myocardial samples were frozen in liquid nitrogen and stored at −80°C until sample preparation. Cardiac tissues from LV and RV were fixed in formalin and embedded in paraffin.

The study conformed with the principles outlined in the Declaration of Helsinki, and the study protocol was approved by the Niguarda Cà Granda Hospital ethics committee. All subjects gave written informed consent to participate in the study.

2.3. Protein and mRNA extraction

The extraction of both protein and mRNA was performed from a single sample using the phenol-guanidine thiocyanate technique [32] as previously described by us [27]. Briefly, RNA was isolated by the Rneasy Fibrous Midi protocol (Qiagen GmbH, Hilden, Germany), and the concentration and purity were determined by the 260–280-nm absorbance, while the integrity was determined by electrophoresis on agarose gel.