Clinical Case Report

Morphological changes in mitochondria during mechanical unloading observed on electron microscopy: a case report of a bridge to complete recovery in a patient with idiopathic dilated cardiomyopathy

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1. Introduction

Left ventricular assist devices (LVADs) are widely used as a bridge to heart transplantation in end-stage heart failure (HF) patients or as destination therapy. In addition, providing hemodynamic support for unloading the LV using an LVAD can facilitate a sufficient myocardial recovery to allow for explantation, known as a “bridge to recovery” (BTR), in some patients. The success of BTR varies significantly across reported studies, presumably due to a variety of factors affecting both clinical and biological outcomes. Biological parameters of cardiac remodeling have been shown to be favorably altered during LVAD treatment, including cardiac hypertrophy, contractile dysfunction, sympathetic denervation, metabolic changes, autophagy, a decreased microvascular density, neurohumoral and cytokine dysregulation, and denaturation of the extracellular matrix [1]. Most such investigations have been directed primarily by volumetric regression and morphometric improvements in cardiomyocytic mitochondria.

The recovery of the cardiac function under mechanical support has not been well documented from a histopathological point of view. We herein report a case of idiopathic dilated cardiomyopathy in which the patient showed a complete recovery of the systolic function following treatment with a left ventricular assist device (LVAD) for deteriorated heart failure. A light microscopic observation showed marked regression of hypertrophic myocytes with significant intracellular vacuolization and scarcity at the time of LVAD implantation after the administration of mechanical support. Furthermore, an electron microscopic observation revealed that these findings were regulated primarily by volumetric regression and morphometric improvements in cardiomyocytic mitochondria.

2. Case report

A 29-year-old male was hospitalized at our institute for refractory HF diagnosed as IDCM (Fig. 1). He had no family history of cardiomyopathy or previous events of HF based on a medical interview. On admission, an electrocardiogram showed sinus tachycardia, with no evidence of intraventricular conduction block and a QRS duration of 104 ms. In addition, a chest X-ray disclosed moderate cardiomegaly and pulmonary congestion, and the initial echocardiogram demonstrated an enlarged LV cavity with an LV end-diastolic dimension of 74 mm, as well as a severely reduced systolic function and LV ejection fraction (EF) of 15% (Fig. 2A). The serum B-type natriuretic peptide (BNP) level was 1159 pg/ml. Despite the administration of intensive pharmacotherapy, including intravenous diuretics, antineurohumoral agents, and inotropic drugs, additional diuretics, antineurohumoral agents, and inotropic drugs, additional support with an intraaortic balloon pump was required due to the patient’s deteriorated hemodynamic state. His condition progressively worsened, with an increase in the serum total bilirubin level to 3.3 mg/dl and a cardiac index of 1.8 l/min/m². We therefore decided to place a temporary pulsatile LVAD (Nipro, Tokyo, Japan) (LVADim) with concomitant intensified pharmacotherapy, including an uptitrated beta-blocker and renin–angiotensin–aldosterone system inhibitors, in combination with incremental cardiac rehabilitation. The patient’s systemic condition and hemodynamic and laboratory data gradually returned to normal in association with improved LV contractions over several months (Fig. 2B).
Finally, he was able to undergo LVAD explantation (LVADex) 6 months after LVADim and was subsequently discharged. He remained free from symptoms, including exertional dyspnea, and his functional activity recovered to baseline, with an LVEF of 65%, according to the regular ambulatory workup performed 6 months after LVADex.

During hospitalization, myocardial tissue specimens were obtained from the apical core of the LV free wall under both LVADim and LVADex. Under LVADex, a sample was obtained approximately 1.5 cm from the operative scar from the LVAD inflow cannula in order to avoid including tissue with characteristics of reactive inflammation and degeneration. Consequently, there were no significant signs of inflammatory cell infiltration in any of the myocardial samples on immunohistological staining with CD3, CD68, or tenasin C. In addition, light microscopy (LM) showed that the cardiomyocytes in the sample obtained under LVADim were severely hypertrophied, thus exhibiting marked intracellular vacuolization and scarcity (Fig. 3A–B). Under treatment with hemodynamic support using LVAD for 6 months, the cardiomyocyte hypertrophy markedly regressed, with a change in diameter from 28±4 μm to 18±4 μm, in addition to discreet intracellular vacuolization and scarcity in the samples obtained during LVADex (Fig. 3E–F). Transmission electron microscopy (EM) (Hitachi H–7650, Tokyo, Japan) of the sample obtained using LVADim showed that the hypertrophied cardiomyocytes contained preserved myofibrils with an increased amount of myocyttoplasm and a significant number of enlarged mitochondria with various morphological abnormalities, such as varying sizes and shapes with disorganized cristae (Fig. 3C–D, obtained from an intermediate zone of the LV wall on LVADim). In the samples obtained from the intermediate zone of the LV wall on LVADex, the fraction of myocyttoplasm was markedly decreased in association with a decrease in number and uniform size of the mitochondria, whereas there were almost no morphological changes in the myofibrils (Fig. 3J–K), in parallel with the LM findings of regressed cardiomyocyte hypertrophy with discreet intracellular vacuolization and scarcity. The statistical analyses conducted using Wilcoxon rank sum test (JMP 10.0 software program for Windows) showed significant decreases in the number of mitochondria (131±52 vs. 75±33/100 μm², P<.05) and the size (0.9±0.6 vs. 0.5±0.3μm, P<.05) and volume ratio of mitochondria/myocyte (35%±11% vs. 20%±10%, P<.05; Adobe Photoshop ver. 11.0.2 and Lumina Vision ver. 3.3.2.0 software programs) on the basis of an observation of 30 randomly selected sections (100 μm²) at a magnification of ×10,000 on LVADim compared with those observed on LVADex, respectively. Regarding intersectional diversity, we compared the endocardium side, the intermediate zone, and the epicardium side of the LV wall on the basis of an observation of 10 randomly selected sections for each side. More significant improvement of mitochondrial denaturation was observed toward the endocardium side (Fig. 3E as endocardium side on LVADim, Fig. 3F as intermediate zone on LVADim, Fig. 3G as epicardium side on LVADim, Fig. 3L as endocardium side on LVADex, Fig. 3M as intermediate zone on LVADex, and

![Fig. 1. Time course of heart failure management including left ventricular assist device (LVAD). Time courses of left ventricular ejection fraction (LVEF), LV end-diastolic dimension (LVEDD), and serum B-type natriuretic peptide (BNP) relative to the administration of drug and mechanical support. Despite the use of intensive treatment, including intravenous diuretics, antineurohumoral agents, and inotropic drugs, and additional support with an intraaortic balloon pump (IABP), the patient’s hemodynamic state progressively deteriorated. After LVAD implantation (LVADim), his systemic condition improved in association with the hemodynamic and laboratory data, gradually accompanied by a recovery in the LV contractions, over several months. Finally, he was able to undergo LVAD explantation (LVADex) 6 months after LVADim.](image1)

![Fig. 2. Echocardiographic improvement during the mechanical support. These figures of the left ventricular (LV) were recorded using B mode (left side) and M mode (right side) from the parasternal long-axis views, respectively. During mechanical support, the LV ejection fraction (LVEF) dramatically improved in association with a decrease in the LV end-diastolic dimension (LVEDD) on LV assist device (LVAD) explantation (B) compared with that observed on LVAD implantation (A).](image2)
Fig. 3. Photomicrographs of serial myocardial biopsies. On left ventricular assist device implantation (LVADim), the light microscopy (LM) showed severely hypertrophied myocytes, with marked intracellular vacuolization and scarcity (A), with mild interstitial fibrosis (B). Electron microscopy (EM) showed an increased number and variety of sizes of mitochondria, with disorganized cristae accompanied by abnormally small and fragmented mitochondria shown in (C) and (D). Conversely, on LVAD explantation (LVADex), the EM showed a regression in cardiomyocyte hypertrophy with discreet intracellular vacuolization and scarcity (H) and increased interstitial fibrosis (I). In contrast, the EM demonstrated a decreased number and a uniform size of mitochondria, with an improvement in the disorganized cristae (J and K). More significant improvement of mitochondrial denaturation was observed toward the endocardium side. The endocardium side (E), the intermediate zone (F), and the epicardium side (G) were obtained during LVADim, whereas the endocardium side (L), the intermediate zone (M), and the epicardium side (N) were obtained during LVADex. The samples in (A) and (H) were stained with hematoxylin and eosin. The samples in (B) and (I) were stained with Masson’s trichrome. The bars in (A), (B), (H), and (I) indicate 100 μm, whereas those in (C) and (J) indicate 5 μm, those in (D) and (K) indicate 1 μm, and those in (E), (F), (G), (L), (M), and (N) indicate 4 μm.

Fig. 3N as epicardium side on LVADex). Fig. 4 shows the comparative results of mitochondrial morphology among three sections. Significant decreases in the number of mitochondria (154±56 vs. 81±29/100 μm², \(P<.05\); Fig. 4A), size (1.2±0.8 vs. 0.5±0.3 μm, \(P<.05\); Fig. 4B), and volume ratio of mitochondria/myocyte (44%±8% vs. 27%±11%, \(P<.05\); Fig. 4C) were seen in the endocardium side on LVADex compared with LVADim, whereas no significant decrease was seen on the epicardium side. The degree of interstitial fibrosis, on the other hand, was increased during the LVAD treatment, with a change in the collagen volume fraction from 3%±2% to 11%±6%.

![Graphs](image-url)

**Fig. 4.** Comparative results of mitochondrial morphology among three sections. Significant decreases in the number of mitochondria (A), size (B), and volume ratio of mitochondria/myocyte (C) were seen on the endocardium side during left ventricular assist device explantation (LVADex) compared with LVAD implantation (LVADim), whereas no significant decrease was seen on the epicardium side. *\(P<.05\), compared with LVAD implantation (LVADim) using Wilcoxon rank sum test.
3. Discussion

The precise mechanism of BTR during LVAD therapy remains unclarified at the detailed biological and clinical level [1]. To the best of our knowledge, this is the first report focusing on ultrastructural changes in the cardiomyocytic mitochondria in a patient with BTR.

Recently, it has become widely noted that LVAD treatment induces the regression of myocyte hypertrophy by unloading the failing heart [1]. Morphologically, a partial improvement in the contractile myofila-
mament following LVAD support has been recognized in some reports [1]; however, few investigations have reported changes in the myocytosplasm during the process of BTR. In addition to regression in the size of myocytes and/or degree of hypertrophy during LVAD sup-
port, prominent cytoplasmic vacuolization with a scarcity of myofibrils was notably diminished in this case. Although such scarcity is originally defined as a decreased or absent number of myofibrils, LM observations may be used to recognize relative disparities between myofibrils and the cytoplasm with respect to the seeming appearance of myofibril scarcity or colliquative myocytosis, even when the absolute volume of myofibrils is preserved, as noted in this case.

In a comparative histopathological study using myocardial tissue samples obtained from patients with cardiomyopathy, the fatal cases involved more prominent fibrosis with less severe vacuolization, while some of the survivors with vacuolization did not exhibit remarkable fibrosis [2]. Although vacuolization is considered to be a result of myofibril degeneration leading to intracellular fibrosis, it may also constitute one of the early changes of the myocardium under certain circum-
stances, such as reactions or adaptation. In patients with ischemic HF, the regional LV wall motion eventually normalizes after coronary by-
pass grafting if colliquative myocytolysis is observed histologically. Furthermore, the findings that myofibrils with colliquative myocytolysis retain enzymes and other proteins indicate the presence of an intact and viable sarcolemma that is not necessarily injured irreversibly with subsequent cell death and/or eventual myocardial fibrosis.

The type of myocardial vacuolization may vary based on the condi-
tions and pathological changes, such as that observed in storage or parasitic diseases, or may occur as an artifact secondary to autolysis or histologic processing. However, with respect to the myocardial tissue on LVAdim in this case, EM did not demonstrate degenerated cardiac myofibrils or the specific findings described above, but rather showed many vacuoles containing intracellular organelles with primarily deformed mitochondria. There have been only a few reports regarding the relevance of mitochondrial morphology to cytoplasmic vacuolization, and little is known about the degree of ultrastructural reversibility of the remodeling process after LVAD. Nevertheless, ultrastructural examinations of myocytes are expected to provide further insight into the findings of LM, such as segmented nuclei, disarrayed myofibrils, and/or deformed mitochondria.

The morphology of mitochondria, which is regulated by a group of fusion and fission proteins, is responsible for changes in cardiomyocytes [3]. In failing hearts, morphological abnormalities in mitochondria include increased numbers and variation in size, destruction of the cristae, and/or the presence of concentric lamellae [3]. Ventricular unloading using a long-term LVAD has been shown to improve the efficiency of cardiomyocytic mitochondrial metabolism, including the potentiation of the endogenous NO-mediated regulation of mitochondrial respira-
tion, leading to a myocardial recovery in patients with end-stage HF. However, there are scarce reports concerning the clinical relevance of morphological alterations in cardiomyocytic mitochondria during LVAD support. There is only one detailed report of EM observation using heart samples obtained before and after LVAD in patients without BTR, leading to subsequent heart transplantation, in which the cardiomyocytic mitochondria appeared larger and were less electron dense in the post-LVAD samples [4], opposite to that noted in the present case. However, they must be derived from original biological differences between myocardial tissue obtained from patients with and without LV reverse remodeling (LVR). In fact, among reported pa-

tients without BTR, the mitochondria in the normal heart resemble those in the pre-LVAD [4].

Alternatively, the detection of a histopathology prone to myocardial inflammation in addition to reduced fibrosis may assist in predicting the potential for BTR [5]. Although the resolution of myocardial inflam-


References


