Mechanical unloading and cell therapy have a synergistic role in the recovery and regeneration of the failing heart

Michael Ibrahim, Christopher Rao, Thanos Athanasiou, Magdi H. Yacoub and Cesare M. Terracciano

INTRODUCTION

Heart failure is the consequence of pathological remodelling of the myocardium after damage due to any cause, and is defined by a cardiac output insufficient to meet the demands of the metabolizing tissues [1]. Heart failure has an estimated prevalence of 3% in the population and an incidence of 1% [2]. End-stage heart failure is associated with up to 50% mortality at 1 year after diagnosis [3]. The gold standard option for patients with end-stage disease is cardiac transplantation [4]. However, this strategy is limited by a shortage of suitable donor hearts, the problems of immunological rejection and the attendant need for lifelong immunosuppression. Consequently, there is a need to revisit our understanding of the physiology of the heart's adaptive and maladaptive mechanisms and develop novel therapeutic strategies.

There are two essential problems in the failing heart: the consequences of the initial injury (loss of myocardium and loss of myocardial function) and the chronic damage due to persistent overload, which is self-maintained (Fig. 1). Although the initial insult may predominantly result in either a loss of cell number (e.g. after myocardial infarction) or function (e.g. after aortic stenosis, familial cardiomyopathy), the pathogenesis of end-stage heart failure implicates both processes. The ideal therapeutic approach consists of removal of the causative insult and restoration of cell number.

This review argues that left ventricular assist devices (LVAD) and stem cell therapy (SCT) have synergistic properties based on shared mechanistic actions (Table 1). This is broadly because (i) both cell and LVAD therapy are able to influence cellular function and number; and (ii) LVAD therapy may enhance the efficacy of cell therapy by promoting favourable conditions for engraftment of cells.

Despite some clinical use of combined SCT and LVAD therapy, the hypothesis that these two approaches have a synergistic role has not been tested rigorously, but there is some evidence that experimental cell transplantation (of syngeneic smooth muscle cells) may improve the cardiac reverse remodelling after unloading is ceased (in a model of LVAD removal) [5]. We discuss shared actions at the systems, organ, tissue and cellular levels. We critically analyse the current clinical experience of the combined use of LVADs and SCT.

STEM CELL THERAPY AND LEFT VENTRICULAR ASSIST DEVICE THERAPY AT THE SYSTEMS LEVEL

The neurohormonal regulation of the cardiovascular system underlies its ability to adapt to changes in demand, and also plays a role under disease conditions. LVADs and SCT individually modulate neurohormonal activation and may have combinatorial benefits. Normalization of the neurohormonal milieu by LVAD therapy may promote the conditions for cell engraftment, which could enhance the effects of SCT.

LVAD therapy reduces plasma catecholamine levels as well as blunting the activation of the renin-angiotensin-aldosterone system [6]. Atrial and B-type natriuretic peptide (BNP) plasma levels are reduced as a function of the reduction in cardiac load [7]. LVAD therapy normalizes the beta-adrenergic system,
the important pro-inflammatory cytokine TNF-α [8], and interleukins 6 and 8 [9–12].

Animal studies suggest that SCT can improve neurohormonal activation in heart failure [13]. In a model of overload-induced right heart failure, the introduction of either human amniotic fluid stem cells or rat adipose tissue stromal vascular fraction GFP-positive cells reduced BNP levels and pro-inflammatory cytokines including TNF-α, which may be responsible for the reduced apoptosis observed. SCT, in this case, also increased anti-inflammatory cytokines, which may then produce beneficial paracrine changes promoting cell engraftment. These changes, apart from promoting cell engraftment, may also independently enhance myocardial reverse remodelling.

The shared mechanism of modulation of neurohormonal and cytokine messengers suggests the possibility that combined use may be synergistic. In this way, LVAD-induced neurohormonal modulation improves cell engraftment which further improves neurohormonal reverse remodelling and enhances myocardial functional improvements.

**Table 1:** Summary of changes after LVAD or SCT

<table>
<thead>
<tr>
<th>Changes after LVAD therapy or SCT</th>
<th>LVAD</th>
<th>SCT</th>
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<tbody>
<tr>
<td>Decreased neurohormones</td>
<td>[6]</td>
<td>[13]</td>
</tr>
<tr>
<td>Decreased cytokines</td>
<td>[7]</td>
<td></td>
</tr>
<tr>
<td>Decreased apoptosis</td>
<td>[50]</td>
<td>[51]</td>
</tr>
<tr>
<td>Decreased whole heart dimensions</td>
<td>[10]</td>
<td>[18]</td>
</tr>
<tr>
<td>Improves LV geometry and CO</td>
<td>[10, 21]</td>
<td>[18]</td>
</tr>
<tr>
<td>Reduces cell size</td>
<td>[27]</td>
<td></td>
</tr>
<tr>
<td>Normalizes beta-adrenergic pathways</td>
<td>[12]</td>
<td></td>
</tr>
<tr>
<td>Alters collagen pattern [or fibrosis, varied reports]</td>
<td>[16]</td>
<td>[19]</td>
</tr>
<tr>
<td>Normalization of the cytoskeleton</td>
<td>[52]</td>
<td></td>
</tr>
<tr>
<td>Improved cellular Ca²⁺ handling</td>
<td>Fig 2</td>
<td>[31]</td>
</tr>
</tbody>
</table>

**STEM CELL THERAPY AND LEFT VENTRICULAR ASSIST DEVICE THERAPY AT THE MYOCARDIAL LEVEL**

**Anatomic changes**

Both LVAD therapy and SCT can induce ventricular reverse remodelling, with diminution of the pathologically enlarged ventricles. LVAD therapy induces reductions in LV dimensions and normalizes ventricular morphology [10]. Other structural changes occur during LVAD support, including changes to the degree and pattern of collagen deposition. Some investigators report increased myocardial fibrosis during mechanical unloading [14], and others report a reduction [15]. Bruggink et al. [16] demonstrated a biphasic change in collagen content in the myocardium with initial expansion of ECM and then a subsequent regression with prolonged unloading.

Data from in vivo and in vitro experiments suggest that cell therapy with mesenchymal stem cells may have beneficial effects on the survival of existing myocardium, promote neovascularization and modulate remodelling of the extracellular matrix [17]. Nelson et al. [18] showed that the introduction of induced pluripotent stem cells [iPSC] in a murine post-myocardial infarction model prevented the dilation of the ventricle, compared to injection of control cells. In addition, a recent clinical study using cardiac stem cells [CSCs] in patients with ischaemic cardiomyopathy shows that SCT can also reduce infarct size [19].

**Functional changes in whole heart function**

LVAD therapy has a complex effect on whole heart contractility, but most trials show improvements in native cardiac function and composite survival endpoints compared to patients.
managed with best medical therapy, as well as improved outcomes for patients who later undergo cardiac transplantation [4]. These can be defined by the indication for LVAD therapy. There are three indications for LVAD implantation; (i) destination therapy where the LVAD is implanted for permanent circulatory support; bridge to transplantation, (ii) where the LVAD is indicated for circulatory support until cardiac transplantation, and (iii) bridge to recovery (BTR), where an LVAD is implanted with the aim of weaning from circulatory assistance as heart failure remission is achieved. BTR results in explantation of the device without recurring to cardiac transplantation. It is in the context of BTR that functional improvements are most relevant. BTR is observed in a low proportion of patients in most series [20]. Functional improvements in the context of LVAD support could be time-dependent, with maximal gains initially and regression of functional improvements over time [21]. This suggests that the combination with other therapies could enhance cardiac recovery.

Cell therapy with mesenchymal stem cells has beneficial effects on the survival of existing myocardium, promotes neovascularization, improves cellular metabolism and contractile function, modulates remodelling of the extracellular matrix and activates native progenitor cells [22]. Encouraging data on global cardiac function have usually come from relatively short 4- to 8-week [23, 24]. However, until recently, assessment of cardiac function beyond 12 weeks does not have demonstrated any significant functional improvement [25]. It has been suggested that the improvement observed with hESC-derived cardiomyocytes is due to paracrine action and this effect of cell therapy is diminished at this stage. It has also been suggested that the failure of hESC-derived grafts, which often remain encapsulated in a thin layer of extracellular matrix components, to fully integrate into the host myocardium may also explain the absence of long-term functional benefit [25]. Injection of iPSC also improves myocardial function, with an approximate doubling of the ejection fraction 4 weeks after myocardial infarction [18]. In a recent clinical study, Bolli et al. [19] showed that the injection of autologous CSCs after surgical revascularization results in sustained improvements in myocardial function, with augmented improvements 1 year after SCT. This exciting study shows that with the identification of SCTs with truly proliferative potential, we are likely to see more robust clinical improvements.

**STEM CELL THERAPY AND LEFT VENTRICULAR ASSIST DEVICE THERAPY AT THE TISSUE ELECTROPHYSIOLOGICAL LEVEL**

Heart failure is associated with whole heart electrophysiological remodelling, with changes to the electrocardiogram, including QT prolongation, and an increase in arrhythmias. The effect of LVAD therapy on arrhythmias is unclear and warrants further studies [26]. The presence of an LVAD means that ventricular arrhythmias do not immediately threaten end organ perfusion, but right heart failure can result in failure to fill the left ventricle and important right-sided symptoms. A change in the burden of arrhythmias during LVAD support could arise due to changes in fibrosis patterns and amount, changes in ion channel expression or as a result of the activity of mechno-electric feedback channels.
There are two mechanisms by which SCT may impact tissue electrophysiology. Primary effects may arise due to paracrine signals which normalize or homogenize tissue electric conduction. Secondary effects may arise due to SCT-induced reverse remodelling by either neovascularization or restoration of normal cardiomyocyte populations. Nelson et al. [18] showed that the QT interval prolongation, which is a part of the myocardial infarction-related pathological remodelling, was partially halted by iPSC therapy, whereas injected fibroblasts did not.

CELLULAR CHANGES AFTER LEFT VENTRICULAR ASSIST DEVICE AND STEM CELL THERAPY

The major change in cellular properties after mechanical unloading is a large reduction in cell size, which has been documented in clinical studies of mechanical unloading [27] and in animal models [28]. The relationship between changes in cell size and function is complex. Increases in cell size are not always associated with dysfunction [29] and regression of cell size does not correlate with functional improvements clinically [27]. Additionally, large reductions in cell size are sometimes associated with dysfunction [30].

The cellular mechanisms which link membrane electric excitation with cellular contraction undergo major remodelling under conditions of chronic overload and heart failure as well as in the reverse remodelling observed after mechanical unloading (Fig. 2). Using ventricular tissue taken from patients pre- and post-LVAD therapy, we demonstrated that patients who show the phenomenon of cardiac recovery during LVAD therapy develop a pattern of specific cellular electrophysiological reverse remodelling which is associated with significant improvements in cardiac function [27]. Importantly, they indicate that the electrophysiological features of heart failure are not irreversible.

We have shown that skeletal myoblasts and bone marrow-derived cell injection in failing myocardium alters the functional properties of the recipient cardiomyocytes, reducing cell hypertrophy and normalizing many features of cell Ca²⁺ cycling [31]. This recovery of function can be explained, at least in part, by the secretion of soluble mediators (paracrine factors) that affect cardiac myocyte function, mirroring the effects of LVAD therapy.

MECHANICAL UNLOADING AND CELL THERAPY FOR REGENERATION

Mechanical unloading per se may stimulate the normally senescent cell cycle of adult cardiomyocytes. It was recently reported that LVAD therapy alters DNA content and increases cell nucleus number, a prerequisite to cell division [32]. A previous study reported decreased cardiomyocyte nuclear size and chromatin density after LVAD therapy [33]. LVADs may also increase the number of circulating bone marrow progenitor cells which could partly explain the functional improvements after LVAD and also indicates that LVADs may promote cardiac regeneration [34]. Suzuki et al. [35] reported that an animal model of mechanical unloading increased the number of stem cells in the myocardium, including Sca-1-positive stem cells and c-kit-positive cells. Furthermore, LVAD therapy acts as a platform for SCT [36]. Several investigators have highlighted the importance of the harsh neurohumoral milieu of the failing heart as a barrier to regeneration.
TABLE 2: Current experience with combined LVAD and SCT

<table>
<thead>
<tr>
<th>Study</th>
<th>Study type</th>
<th>Cell type</th>
<th>Clinical outcome</th>
<th>Structural finding</th>
<th>Functional finding</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Db et al. [53]</td>
<td>Phase I</td>
<td>Skeletal myoblast, $3 \times 10^8$, 6 patients, 4 underwent heart TX,</td>
<td>Persistence of grafted cells in scarred myocardium</td>
<td>Shows LVAD and SCT combination is feasible in ischemic patients. Persistence of grafted cells in scarred myocardium</td>
<td>Reduced fibrosis, new vessel formation. Reduced BNP, increased EF improved from 6.4 to 40%.</td>
<td>Although lacking a control, these improvements were documented on a baseline of LVAD support.</td>
</tr>
<tr>
<td>Pagani et al. [54]</td>
<td>Phase I</td>
<td>Skeletal myoblast, $3 \times 10^8$, 5 patients, 1 death, three Tx,</td>
<td>Cell survival and differentiation in scar.</td>
<td>Shows LVAD and SCT combination is feasible in ischemic patients.</td>
<td>New vessel formation</td>
<td>New vessel formation feasibility, with survival of the graft.</td>
</tr>
<tr>
<td>Anastasiadis and Antonitsis [55]</td>
<td>Case series</td>
<td>Autologous bone marrow, 1 patient, SCT 3 month post-LVAD.</td>
<td>Reduced fibrosis, new vessel formation.</td>
<td>Although lacking a control, these improvements were documented on a baseline of LVAD support.</td>
<td>Increased perfusion, 1 month after SCT.</td>
<td>Increased perfusion in one patient.</td>
</tr>
<tr>
<td>Miyagawa et al. [56]</td>
<td>Case report</td>
<td>BMNCs after LVAD 1 patient, on LVAD for 99 days and explanted cells.</td>
<td>Reduced fibrosis, new vessel formation.</td>
<td>Although lacking a control, these improvements were documented on a baseline of LVAD support.</td>
<td>Increased perfusion, 1 month after SCT.</td>
<td>Increased perfusion in one patient.</td>
</tr>
<tr>
<td>Gojo et al. [57]</td>
<td>Case report</td>
<td>BMNCs and concomitant hESC after LVAD</td>
<td>Reduced fibrosis, new vessel formation.</td>
<td>Although lacking a control, these improvements were documented on a baseline of LVAD support.</td>
<td>Increased perfusion, 1 month after SCT.</td>
<td>Increased perfusion in one patient.</td>
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CURRENT EXPERIENCE OF DIRECT COMBINATION OF LEFT VENTRICULAR ASSIST DEVICE AND STEM CELL THERAPY

LVADs reduce LV wall tension and improve perfusion, which could promote more favourable conditions for survival of transplanted cells. Moreover, the evidence presented for each level of action shows that LVAD therapy and SCT might have a greater efficacy when combined than when used singly (Fig. 3). Table 2 presents all the studies currently available where SCT and LVAD have been combined. Because of the evidence of functional improvements after the transplantation of bone marrow mononuclear cells (BMNCs) and after LVAD therapy, Nasseri et al. [43] hypothesized that the injection of BMNCs concomitantly with LVAD therapy would enhance the rate of weaning from LVADs due to augmented cardiac recovery. This study found no evidence for enhanced cardiac repair under these circumstances, but concluded that other cell therapies should be trialled. It is apparent that the current available evidence is limited, with a total of 25 patients across all the studies and no control groups. All the studies conclude that this issue requires further attention, and our intention in drawing together the evidence in this paper...
is to argue for larger studies with a control group. It is also apparent that the two studies (both case reports) which document substantial increases in function employed a study protocol where LVAD therapy was commenced first and supported the patients for a period of approximately 3 months and that SCT was then introduced. This could be because the LVAD had time to normalize the myocardial structure and function, and thereby provide a better substrate for SCT.

The successful application of these therapies in the future depends on refining them individually but also maximizing their possible combinatorial efficacy. For SCT, this includes identifying the best cell population, the optimal method of harvesting, expansion and delivery and importantly the best time or context of delivery. For LVAD therapy, this involves identifying the best devices, the optimal time for implantation (at what disease stage) and for how long and at what pump speed. Pharmacological augmentation of LVAD-induced reverse remodelling, using the beta-agonist Clenbuterol, has been documented by our group and can greatly increase the rate of LV AD explanting, using the beta-agonist Clenbuterol, has been documented [4]. This has not currently been shown in a larger patient population by the multicentre clinical trial, currently underway (HARPS trial, NCT00585546). These questions require the attention of a randomized, multicentre double-blind trial, but the heterogeneity in patient age, clinical status, device and choice of optimal cell population makes this practically extremely difficult. Clinical studies continue to document the efficacy of LVAD therapy in different scenarios [45, 46]. Improvements in the delivery and our understanding of the biology of SCT will continue to increase the scope for combined SCT and LVAD use [47–49].

CONCLUSIONS

New therapies for chronic, end-stage heart failure include LVADs which are used clinically to sustain the circulation, but promote reverse remodelling and may induce myocardial recovery. Cell therapy is aimed at introducing cells to replace those lost in the failing heart, but there is still limited evidence that this is effective. Mechanical unloading changes the hostile environment of the failing heart, and normalizes the physiological properties of the surviving myocardium. The use of cells with significant cardiomyogenic potential in combination with mechanical unloading may synergistically improve the efficacy of both therapies in the failing heart.

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REFERENCES


