The impact of left ventricular assist device-induced left ventricular unloading on the myocardial renin–angiotensin–aldosterone system: therapeutic consequences?

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Aims

Angiotensin-converting enzyme inhibitors (ACE-Is) prevent the rise in myocardial angiotensin II that occurs after left ventricular assist device (LVAD) implantation, but do not fully normalize cardiac function. Here, we determined the effect of LVAD implantation, with or without ACE-Is, on cardiac renin, aldosterone, and norepinephrine, since these hormones, like angiotensin II, are likely determinants of myocardial recovery during LVAD support.

Methods and results

Biochemical measurements were made in paired LV myocardial samples obtained from 20 patients before and after LVAD support in patients with and without ACE-I therapy. Pre-LVAD renin levels were 100 normal and resulted in almost complete cardiac angiotensinogen depletion. In non-ACE-I users, LVAD support, by normalizing blood pressure, reversed this situation. Cardiac aldosterone decreased in parallel with cardiac renin, in agreement with the concept that cardiac aldosterone is blood-derived. Cardiac norepinephrine increased seven-fold, possibly due to the rise in angiotensin II. Angiotensin-converting enzyme inhibitor therapy prevented these changes: renin and aldosterone remained high, and no increase in norepinephrine occurred.

Conclusion

Although LV unloading lowers renin and aldosterone, it allows cardiac angiotensin generation to increase and thus to activate the sympathetic nervous system. Angiotensin-converting enzyme inhibitors prevent the latter, but do not affect aldosterone. Thus, mineralocorticoid receptor antagonist therapy during LVAD support may play a role in further promoting recovery.

Keywords

Left ventricular assist device • Renin–angiotensin–aldosterone system • Catecholamine • Collagen • Heart failure • Reverse remodelling

Introduction

Heart failure is one of the most common causes of death in the developed world. Despite modern pharmacological treatment, mortality of advanced heart failure is higher than that of breast or colon cancer.¹ Left ventricular assist devices (LVADs) can improve both short-term and intermediate mortality in patients with end-stage heart failure.² Initial reports suggested that chronic unloading of the failing heart provided by LVADs can result in the recovery of cardiac function and allow explantation of the LVAD device in a very limited number of cases (so-called ’bridge-to-recovery’).³–⁵ A vast array of functional, biochemical, and molecular data support the concept of ’recovery’ of the failing heart during LVAD support.⁶ Nonetheless, the clinical promise of bridge-to-recovery in the majority of LVAD-supported patients has not been reproduced in more recent clinical trials.⁷–⁹

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In addition, LVAD recovery data are somewhat controversial. One group was able to show an LVAD weaning rate of 73.3% with the use of the β2-adrenoceptor agonist clenbuterol combined with β-blockers, angiotensin-converting enzyme inhibitors (ACE-I), and mineralocorticoid receptor antagonism; another group could not wean a single patient from the device by using a similar drug protocol. In any event, the recovery of LV function could not be accomplished by using a single agent.10 We recently showed that LVAD support modifies the extracellular matrix (ECM) in a potentially detrimental way.11 There is increased myocardial collagen deposition, increased collagen cross-linking, increased myocardial stiffness, and changes in the balance between matrix metalloproteinases (MMPs) and its tissue inhibitor (TIMP). Although concomitant treatment with ACE-Is during LVAD support ameliorated these effects in LV myocardium, most likely by reducing cardiac angiotensin (Ang) II levels (Figure 1), recovery of ventricular function was still not complete.12 Thus, it is important that other potential therapeutic targets be pursued to enhance myocardial recovery. As prior investigations have been limited to the examination of tissue levels of Ang I and II, it is next pertinent to examine the impact of LVAD support on the other components of the myocardial renin–angiotensin–aldosterone system (RAAS) as well as myocardial catecholamine stores, all of which are largely unknown. As these neurohormonal systems also regulate cell growth and fibrosis, they are likely to be important in ECM remodelling and in determining myocardial recovery during LVAD support. Therefore, it was the aim of the present study to determine the effect of LVAD implantation, with or without ACE-Is, on cardiac RAAS parameters that had not been determined before (renin, prorenin, angiotensinogen, and aldosterone) as well as catecholamines (norepinephrine, epinephrine, and dopamine).

Methods

Collection of cardiac tissue samples and haemodynamic parameters

This study was performed according to the guidelines of the Declaration of Helsinki. All procedures involving human tissue use were approved by the Institutional Review Board of the New York Presbyterian Medical Center and the Erasmus Medical Center. Myocardial tissue samples were obtained from the LV apex at the time of LVAD implantation (pre-LVAD) and subsequently at the time of cardiac transplantation (pre-Tx) following LVAD support. We included every LVAD patient implanted between June 1998 and October 2001, provided that (i) they gave informed consent to this study, (ii) they reached heart transplantation (allowing us to receive the explanted heart for further analysis), and (iii) sufficient material was available for this study. Patients who died during LVAD support were excluded. This approach resulted in a total of 20 patients, a number that was found to be sufficiently large to allow clinically significant conclusions in previous LVAD studies.11,12 All samples were stored at −70°C. Haemodynamic parameters were obtained as described earlier.11,12

Biochemical measurements

Cardiac tissue was homogenized in 0.01 mol/L phosphate buffer, pH 7.4, containing 0.15 mol/L NaCl, and the homogenates were used to measure renin, total renin (i.e. renin plus prorenin), angiotensinogen, aldosterone, norepinephrine, epinephrine, and dopamine. Renin was measured by antibody-trapping enzyme-kinetic assay.13 Total renin was measured by enzyme-kinetic assay after conversion of prorenin to renin by acidification.13 Angiotensinogen was measured as the maximum quantity of Ang I that was generated during incubation with excess recombinant renin.13 Aldosterone was measured by solid-phase radioimmunoassay.14 Norepinephrine, epinephrine, and dopamine were measured by high-performance liquid chromatography with fluorimetric detection after derivatization with the selective fluorogenic agent 1,2-diphenylethlenediamine.15

Data analysis

Owing to the limited sample size, not all biochemical measurements could be performed in all samples in a paired manner. The figures show the individual data points in maximally 13 paired non-ACE-I users and 7 ACE-I users. Differences between pre-LVAD and pre-Tx samples were evaluated by applying Student’s t-test on paired samples only, after log transformation of the data. A χ²-test was used for categorical variables. Univariate linear associations between renin, blood pressure, and aldosterone were assessed by calculating Pearson’s coefficient of correlation. All tests were two-sided, and statistical significance was accepted at P < 0.05. The statistical software package SPSS 14.0 (SPSS Inc., Chicago, IL, USA) was used for the analysis.

Results

Patient population and medications

Paired myocardial samples from 20 patients obtained at the time of LVAD implantation (pre-LVAD) and subsequently at the time of cardiac transplantation (pre-Tx) were included in this analysis. All patients were on LVAD support with a pulsatile mechanical pump (HeartMate VE LVAD, Thoratec Corp., Pleasanton, CA,
USA). The duration of LVAD support ranged from 18 to 360 days (median, 45 days). Age ranged from 17 to 65 years (mean ± SD 52 ± 12 years), and 85% were male. Six patients (30%) were diagnosed with idiopathic dilated cardiomyopathy and 14 (70%) with ischaemic cardiomyopathy.

Pharmacological treatments at the time of LVAD implantation and cardiac transplantation are summarized in Table 1. Almost all patients were on inotropic support at the time of LVAD implantation, and these drugs were used in only one patient at the time of cardiac transplantation. The use of β-blockers, ACE-Is, and mineralocorticoid receptor antagonists was low in this cohort. Table 2 shows the haemodynamic parameters pre-LVAD implantation and during LVAD support prior to cardiac transplantation. As expected, cardiac output was severely reduced, and pulmonary pressures were elevated pre-LVAD implantation; LVAD implantation virtually normalized these parameters.

**Myocardial tissue renin–angiotensin–aldosterone system components**

Cardiac tissue may contain both renin and its inactive precursor, prorenin. Prorenin can only be measured enzyme-kinetically after its conversion to renin. In 12 samples (6 pre-LVAD and 6 pre-Tx), we quantified the Ang I-generating activity, as a measure of renin activity, both before and after in vitro conversion of prorenin to renin. In these samples, the Ang I-generating activity before prorenin activation was 100 ± 21% of the activity after prorenin activation. Thus, prorenin–renin conversion in vitro did not increase Ang I-generating activity. This suggests that the prorenin levels in these cardiac tissue samples were negligible. Figures 2–4 and Table 3 therefore only show cardiac renin, measured in acidified samples by enzyme-kinetic assay.

To verify that the Ang I-generating activity was truly due to renin, parallel enzyme-kinetic measurements were performed in the presence of the renin inhibitor, aliskiren, at a concentration (10 μmol/L) that fully blocks human renin. Aliskiren, at this concentration, blocked 94 ± 6% of the Ang I-generating activity in acidified (n = 20) samples, confirming that most, if not all, Ang I-generating activity was truly due to renin. Based on this observation, data were not corrected for non-aliskiren-inhibitable Ang I-generating activity.

In subjects not using ACE-Is (n = 13), LVAD implantation reduced cardiac renin (P < 0.001) and aldosterone (P = 0.006), without altering cardiac angiotensinogen significantly (Figure 2 and Table 3). The decrease in cardiac renin correlated inversely with the rise in blood pressure in non-ACE-I users (Figure 3, P = 0.06), suggesting that haemodynamic improvement was the major cause for the decrease in cardiac renin. The strong correlation between cardiac renin and cardiac aldosterone (Figure 4, left panel; P < 0.01) in these subjects suggests that renin-dependent angiotensin generation is a major determinant of cardiac aldosterone.

In subjects using ACE-Is (n = 7), cardiac renin did not decrease after LVAD implantation (Figure 2 and Table 3). Moreover, no significant changes in cardiac aldosterone or angiotensinogen occurred in these subjects (Figure 2), and cardiac renin and aldosterone levels were unrelated (Figure 4, right panel).

Analysis of co-variance, coding pre-LVAD and pre-Tx as categorical dummy variables, showed that the relationships between blood pressure and cardiac renin (Figure 3) and between cardiac renin and aldosterone (Figure 4, left and right panels) were independent of LVAD (P = 0.31, 0.12, and 0.12, respectively).

**Myocardial tissue catecholamines**

Cardiac norepinephrine levels increased almost seven-fold following LVAD implantation in subjects without ACE-I therapy (Figure 5 and Table 3; P = 0.004). No such increase was observed in ACE-I users. Cardiac dopamine levels modestly decreased after LVAD implantation in non-ACE-I users (Figure 5 and Table 3; P = 0.014), but not in ACE-I users. Epinephrine levels were identical in both groups and did not change significantly after LVAD implantation.

**Discussion**

The RAAS plays an important role in the myocardial remodelling process in cardiac diseases.16 Large studies have shown protective effects of blocking the RAAS.17,18 It is now widely accepted that Ang II is generated at cardiac tissue sites19,20 and acts as an autocrine–paracrine modulator of cardiac function and structure.21 The renin and angiotensinogen required for such local synthesis are taken up from blood,22–24 whereas ACE and the angiotensin

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**Table 1** Medication at the time of left ventricular assist device implantation and cardiac transplantation

<table>
<thead>
<tr>
<th></th>
<th>Pre-LVAD</th>
<th>Pre-Tx (on LVAD support)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inotropes</td>
<td>19 (95%)</td>
<td>1 (5%)</td>
<td>0.001</td>
</tr>
<tr>
<td>β-blocker</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
<td>1.00</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>5 (25%)</td>
<td>7 (35%)</td>
<td>0.490</td>
</tr>
<tr>
<td>Mineralocorticoid</td>
<td>5 (25%)</td>
<td>2 (10%)</td>
<td>0.212</td>
</tr>
<tr>
<td>receptor antagonist</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>16 (80%)</td>
<td>4 (20%)*</td>
<td>0.001</td>
</tr>
</tbody>
</table>

LVAD, left ventricular assist device; Tx, cardiac transplantation.

**Table 2** Haemodynamic parameters at the time of left ventricular assist device implantation and cardiac transplantation (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Pre-LVAD</th>
<th>Pre-Tx (on LVAD support)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO (l/min)</td>
<td>3.84 ± 1.10</td>
<td>5.21 ± 1.18</td>
<td>0.044</td>
</tr>
<tr>
<td>RAP (mmHg)</td>
<td>11.4 ± 5.1</td>
<td>9.5 ± 3.4</td>
<td>0.214</td>
</tr>
<tr>
<td>MPAP (mmHg)</td>
<td>33.0 ± 12.8</td>
<td>20.9 ± 2.7</td>
<td>0.045</td>
</tr>
<tr>
<td>PAD (mmHg)</td>
<td>23.1 ± 8.0</td>
<td>14.0 ± 1.7</td>
<td>0.041</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>73.8 ± 9.3</td>
<td>90.7 ± 12.9</td>
<td>0.037</td>
</tr>
</tbody>
</table>

LVAD, left ventricular assist device; Tx, cardiac transplantation; CO, cardiac output; RAP, right atrial pressure; MPAP, mean pulmonary arterial pressure; PAD, pulmonary diastolic pressure; MAP, mean arterial blood pressure.
receptors are expressed in the heart. Circulating, kidney-derived renin enters cardiac tissue sites via diffusion and/or binding to renin receptors, whereas circulating, liver-derived angiotensinogen diffuses into cardiac interstitial fluid. Unexpectedly, renin binding to renin receptors not only facilitates local angiotensin generation, but also results in direct, angiotensin-independent effects such as extracellular signal-regulated kinase phosphorylation, transforming growth factor-β1 release, and increases in plasminogen-activator inhibitor-1, fibronectin, and collagen. As such, it may contribute directly to cardiac fibrosis and remodelling. Initial studies suggested that aldosterone may also be formed locally within the heart, but more recent data do not support this concept. Thus, most, if not all, cardiac aldosterone originates in blood. The cardiac effects of aldosterone include oxidative stress, inflammation, fibrosis, arrhythmias, and hypertrophy.

Left ventricular assist device support unloads the end-stage failing heart and normalizes circulating neurohormonal factors, thus allowing many macroscopic, microscopic, biochemical, and molecular properties of the myocardium to undergo a process of normalization, collectively referred to as reverse remodelling. However, the ECM undergoes continued detrimental remodelling beyond that observed in heart failure alone. Specifically, we recently showed that LVAD-induced unloading leads to increased collagen deposition, increased collagen cross-linking, and changes in the MMP/TIMP ratio that favours increased collagen deposition. These changes were associated with increased myocardial stiffness. Importantly, the use of an ACE-I (which reduces myocardial tissue Ang II: Figure 1), in combination with mechanical unloading, positively corrected this detrimental ECM remodelling. This dual control by mechanical and hormonal factors was revealed by the fact that ACE-Is prevented the ECM remodelling in the LV, but not in the right ventricle of the same hearts. However, no data were available regarding the myocardial tissue levels of RAAS components other than Ang I or II during LVAD support. This is the first study systematically analysing these components.

Figure 2 Myocardial renin, angiotensinogen, and aldosterone levels before [pre-left ventricular assist device (LVAD)] and after [pre-cardiac transplantation (Tx)] LVAD implantation in patients without (top panels) and with (bottom panels) angiotensin-converting enzyme inhibitor medication. Black circles represent individual measurements, and open circles represent the geometric mean. *P < 0.001 (renin) or P = 0.006 (aldosterone) before vs. after.
of the RAAS in paired myocardial samples pre- and post-LVAD implantation.

In view of prior studies showing that the recovery of different aspects of myocardial properties occurs with different time courses during LVAD support, we first explored whether there was any time dependence of recovery of the biochemical markers examined in the present study. The duration of LVAD support ranged from 18 to 360 days and was reasonably balanced between the groups. However, no time dependence of the changes in RAAS components could be identified. One explanation for this is that the recovery of these parameters is very quick. This is not unreasonable, as we previously identified that the recovery of gene expression of specific genes of interest (e.g. SERCA2a) was essentially complete within 15–30 days. However, it must be acknowledged that the sample size is too low to make definitive conclusions.

Renin levels in the pre-LVAD heart reported in this study are the highest ever recorded in human myocardial tissue. On average, cardiac renin levels were approximately 100 times higher than those in normal hearts. Taking into consideration the fact that cardiac renin, even under pathological conditions, is kidney-derived, the most likely explanation for this rise is the low blood pressure in these patients with end-stage heart failure, resulting from their low cardiac output (Table 2). A decrease in blood pressure (leading to a decrease in renal perfusion) is a potent stimulator of renal renin synthesis and release. Chronic renin stimulation causes more prorenin to be converted to renin, leading to an increased renin/prorenin ratio in plasma. This most likely explains the absence of prorenin in pre-LVAD hearts, as opposed to the situation in normal and failing human hearts where prorenin can be detected. On the basis of this concept, one would expect a (near) normalization of blood pressure to rapidly diminish the cardiac (and plasma) renin levels. This is exactly what happened after LVAD implantation: cardiac renin levels decreased by almost 90%, to levels previously observed in subjects with end-stage heart failure. Cardiac aldosterone decreased in parallel with cardiac renin, in full agreement with the idea that cardiac aldosterone is blood-derived and that a decrease in renal renin release would result in reduced circulating Ang II and aldosterone levels. However, in apparent contrast with this observation, cardiac Ang I and II levels increased 5–10-fold post-LVAD implantation. The most likely explanation for this phenomenon is that at the tremendously high renin levels in the pre-LVAD situation, cardiac angiotensinogen levels are depleted prior to LVAD support, so that cardiac Ang I (and II) levels are low instead of high. Such decreases have been noted before in animals treated with high doses of ACE-Is. Indeed, the cardiac angiotensinogen levels in the pre-LVAD hearts in this study (expressed per gram wet weight) were <5% of the angiotensinogen levels that normally occur in blood plasma. Clearly, therefore, at such high renin levels, it is no longer possible to match the rapid metabolism of angiotensinogen in cardiac tissue sites by either increased uptake from blood or local synthesis of angiotensinogen. Only by lowering renin, can cardiac angiotensin generation increase again, as was the case post-LVAD in non-ACE-I users.

This situation is different in subjects using ACE-Is. These drugs lower Ang II levels, which results in a rise of renin due to interference with the negative feedback loop between Ang II and renal renin synthesis. Consequently, renin will remain high, despite the blood pressure normalization following LVAD implantation. In fact, in the present study, there was no significant decrease in renin in the ACE-I users, in full agreement with our previous observation that cardiac Ang II in ACE-I users did not increase post-LVAD. From this point of view, it is not surprising that LVAD support also did not alter cardiac aldosterone: the change in renal renin release apparently was too modest to lower the source of cardiac aldosterone (namely, aldosterone in the
Figure 5  Myocardial norepinephrine, epinephrine, and dopamine levels before [pre-left ventricular assist device (LVAD)] and after [pre-cardiac transplantation (Tx)] LVAD implantation in patients without (top panels) and with angiotensin-converting enzyme inhibitor medication. Black circles represent individual measurements, and open circles represent the geometric mean. *P = 0.004 (norepinephrine) or P = 0.014 (dopamine) before vs. after.

Table 3  Myocardial renin, angiotensinogen, aldosterone, norepinephrine, epinephrine, and dopamine levels before [pre-left ventricular assist device (LVAD)] and after [pre-cardiac transplantation (Tx)] LVAD implantation in patients without and with angiotensin-converting enzyme inhibitor medication

<table>
<thead>
<tr>
<th>No ACE-Is</th>
<th>ACE-Is</th>
</tr>
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<tbody>
<tr>
<td>Pre-LVAD</td>
<td>Pre-Tx</td>
</tr>
<tr>
<td>Renin (fmol Ang I/g min)</td>
<td>8668 (694–132 729)</td>
</tr>
<tr>
<td>Angiotensinogen (pmol/g)</td>
<td>17 (1–98)</td>
</tr>
<tr>
<td>Aldosterone (pg/g)</td>
<td>13 793 (2340–41 065)</td>
</tr>
<tr>
<td>Norepinephrine (pg/g)</td>
<td>28 632 (3894–84 735)</td>
</tr>
<tr>
<td>Epinephrine (pg/g)</td>
<td>975 (288–4260)</td>
</tr>
<tr>
<td>Dopamine (pg/g)</td>
<td>1671 (243–8403)</td>
</tr>
</tbody>
</table>

LVAD, left ventricular assist device; Tx, cardiac transplantation. Data are geometric mean and range.
circulation). One might even argue that circulating aldosterone in ACE-I-treated subjects is unlikely to depend on Ang II at all, so that lowering renin release will not alter aldosterone. The lack of a relationship between cardiac renin and cardiac aldosterone in ACE-I users (Figure 4, right panel) supports this view. Importantly, despite the fact that cardiac renin and aldosterone remained elevated during LVAD support, the ACE-I users displayed a decreased myocardial collagen content and myocardial stiffness. This indicates that the deleterious direct, angiotensin-independent effects of renin and aldosterone on growth and remodelling are of less importance than the reduction in Ang II.

Finally, in line with the well-known positive interaction between Ang II and norepinephrine, the increased cardiac Ang II levels in non-ACE-I users during LVAD support were accompanied by a seven-fold rise in cardiac norepinephrine content. This was not observed in patients using ACE-Ils. High levels of norepinephrine lead to cardiac fibrosis and apoptosis, and this could therefore, in part, contribute to the increased cardiac fibrosis and ongoing myocardial loss, following LVAD support in the absence of ACE-Ils.

Conclusion

Left ventricular assist device-induced LV unloading influences the RAAS significantly; although it decreases renal renin release (and thus, presumably, the plasma levels of Ang II and aldosterone), it allows cardiac angiotensin generation to increase. The latter most likely relates to the rate-limiting quantities of angiotensinogen in cardiac tissue (Figure 6). A decrease in circulating aldosterone (the main source of cardiac aldosterone) would explain why cardiac aldosterone decreased in parallel with cardiac renin. However, cardiac norepinephrine increased seven-fold, possibly due to the rise in Ang II generated at cardiac tissue sites. Both agonists are likely determinants of the increased myocardial fibrosis and stiffness following LVAD support. Importantly, the use of an ACE-I not only prevented the increase in angiotensin II and norepinephrine, but also ameliorated myocardial fibrosis and stiffness. Nevertheless, it did not result in a complete recovery of ventricular function. As these beneficial effects occurred in the absence of a change in cardiac aldosterone, additional improvement might be expected by applying mineralocorticoid receptor antagonists in addition to ACE-Ils during LVAD support. Obviously, this conclusion should be viewed with care, given the low patient number investigated in this study. Additionally, β-blockers might further suppress sympathetic activity. It is interesting to note, however, that both ACE-Ils and mineralocorticoid receptor antagonist therapy were employed (in addition to other agents) by Birks et al. in their series of patients, which resulted in a ~70% rate of myocardial recovery allowing LVAD explantation. Additional studies should prospectively test the contribution of these various drugs to myocardial recovery during prolonged mechanical unloading by LVADs.

Conflict of interest: None declared.

References

1. Stewart S. Prognosis of patients with heart failure compared with common types of cancer. Heart Fail Monit 2003;8:87–94.


