Left ventricular assist device support reverses altered cardiac expression and function of natriuretic peptides and receptors in end-stage heart failure

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Abstract

Objective: Atrial (ANP) and B-type natriuretics peptides (BNP) via their guanylyl cyclase-A (GC-A) receptor not only regulate arterial blood pressure and volume but also exert local antihypertrophic, antifibrotic and lusitropic effects in the heart. To elucidate whether cardiac hypertrophy/insufficiency and reversal is associated with changes in the local responsiveness to NPs, we compared the mRNA expression of ANP, BNP and receptors and the responsiveness of GC-A to ANP in left ventricular tissue obtained from 10 patients with congestive heart failure (CHF) before and after hemodynamic unloading by left ventricular assist device (LVAD) support.

Methods and results: Quantitative “real time” RT-PCR demonstrated that the mRNA expression levels of ANP, BNP and the NP-metabolizing NPR-C receptor were both markedly increased in human failing hearts. GC-A mRNA expression levels were not different from nonfailing hearts, but cGMP production by GC-A in response to ANP was nearly abolished. Reversal of cardiomyocyte hypertrophy during LVAD support was accompanied by normalization of ANP, BNP and NPR-C mRNA levels and a significant recovery of GC-A responsiveness to ANP.

Conclusion: In CHF patients, increased local clearance by NPR-C receptors and diminished responsiveness of cardiac GC-A might impair the local antihypertrophic effects of natriuretic peptides and contribute to the progression of cardiac hypertrophy and insufficiency. Reverse remodeling during LVAD support reverses these changes and can thereby recuperate the local protective effects of ANP and BNP.

Keywords: Natriuretic peptide; Heart failure; Transplantation

1. Introduction

Cardiac atrial (ANP) and B-type natriuretics peptides (BNP) not only act as circulating endocrine factors to maintain arterial blood pressure and volume homeostasis but also as local auto/paracrine antihypertrophic (ANP) and antifibrotic (BNP) cardiac factors [1]. Thus, targeted over-expression of the ANP/BNP receptor guanylyl cyclase-A (GC-A) in cardiomyocytes exerts antihypertrophic effects in vivo [2]. Conversely, mice with conditional, selective deletion of GC-A in cardiomyocytes have normal arterial blood pressure levels, and despite this, significant cardiac hypertrophy and impaired diastolic relaxation [3]. Notably, BNP-deficient mice do not have hypertension or cardiac hypertrophy but are susceptible to cardiac fibrosis [4]. Taken together, these observations in monogenetic mouse models indicate that NP/GC-A/cGMP signaling not only protects the heart from pressure and volume overload, but
also provides a local cell-growth moderating and possibly lusitropic circuit within the heart itself. In particular, this system can counteract Angiotensin-II-mediated excessive remodeling [5].

Patients with cardiac hypertrophy and/or congestive heart failure (CHF) have elevated cardiac and plasma levels of ANP and BNP, with these peptide levels being highly related to the severity of the disease. However, diuresis/natriuresis, vasodilatation as well as vascular cGMP synthesis in response to exogenous ANP or BNP are markedly attenuated, indicating a down-regulation or impaired receptor or postreceptor responsiveness of GC-A in peripheral tissues of CHF patients [6–8]. Increased peripheral expression of NPR-C receptors, mediating enhanced metabolic clearance of NPs, may also be involved [9,10]. It is not known whether attenuation of the endocrine ANP effects in CHF patients is accompanied by local cardiac alterations of NP/GC-A/NPR-C signaling. This is important because our recent studies in mice with cardiomyocyte-restricted deletion of GC-A indicate that an inhibition of the local cardiac ANP effects facilitates or can even initiate cardiac hypertrophy and diastolic dysfunction [3]. Using “real-time” quantitative RT-PCR and stimulation of cGMP production, in the present study, we determined the level of transcripts of both the biologically active GC-A and the NP-metabolizing NPR-C receptors as well as ANP-stimulated GC-A activity in left ventricular tissue obtained from patients with CHF before and after long-term hemodynamic unloading. Investigation of paired heart tissue obtained immediately after left ventricular assist device (LVAD) implantation and later during cardiac transplantation provides the unique opportunity to study the effects of volume/pressure unloading within the same patient. Compared with “nonfailing” hearts, the cardiac GC-A/NPR-C ratios and ANP-stimulated GC-A activity were markedly decreased in both ischemic heart disease and dilated cardiomyopathy. Partial reversal of cardiac hypertrophy during LVAD support was accompanied by normalization of cardiac ANP/BNP expression and GC-A/NPR-C ratios and a significant recovery of GC-A responsiveness to ANP. We conclude that chronic hemodynamic unloading by LVAD reverses altered cardiac gene expression and function of NP and their receptors in end-stage heart failure and thereby might reintegrate at least in part their local protective actions.

2. Methods

2.1. Patient material

Paired transmural myocardial tissue from 10 patients with terminal heart failure (five with dilated cardiomyopathy and five with ischemic heart disease) was sampled from the left ventricular apex at the time of LVAD implantation (pre-LVAD) and removal before transplantation (post-LVAD) (Table 1). The mean duration of LVAD support was 210 days (range 110–295 days). The material used in the study is routinely removed during surgery (both during LVAD implantation and at the time of heart transplantation). Only tissue assessed as viable by macroscopic examination was taken to avoid sampling of scar tissue. Control tissue was taken from the left ventricle of unused donor hearts (n=8 “nonfailings”). One part of the samples was snap frozen in liquid nitrogen and processed for mRNA and GC-A activity determinations. The other part was fixed in 4% formalin for morphometrical analyses. The investigation conforms to the principles outlined in the Declaration of Helsinki and was approved by the local ethics committee.

2.2. Morphometrical analysis

Periodic acid Schiff (PAS)-stained slides were used to determine cardiomyocyte diameter. By evaluating 100 cells

### Table 1

Clinical parameters of the CHF patients and cardiac morphometry

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Diagnosis</th>
<th>LVAD duration (days)</th>
<th>Medication before LVAD</th>
<th>Medication after LVAD</th>
<th>Difference after LVAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51</td>
<td>DCM</td>
<td>201</td>
<td>Dob, BB, S, AC</td>
<td>BB, S, AC</td>
<td>n.a.</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
<td>DCM</td>
<td>150</td>
<td>Dob, ACE, Dg, BB, AC</td>
<td>ACE, BB, AC</td>
<td>n.a.</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>DCM</td>
<td>228</td>
<td>Dob, ACE, Du, AC</td>
<td>ACE, BB, AC</td>
<td>−25 −2 −5.46 1.98</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>DCM</td>
<td>249</td>
<td>Dob, ACE, Dg, BB, Du, S</td>
<td>ACE, Dg, BB, Du, S, AC</td>
<td>8 0 3.23 0.55</td>
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<tr>
<td>5</td>
<td>42</td>
<td>IHD</td>
<td>175</td>
<td>ACE, Dg, BB, Du, S, AC</td>
<td>ACE, Dg, BB, Du, AC</td>
<td>−5 0 −0.35 −3.55</td>
</tr>
<tr>
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<td>45</td>
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<td>146</td>
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<td>ACE, Dg, BB,N,AC</td>
<td>−1 −1 −3.13 0.71</td>
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<tr>
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<td>18</td>
<td>DCM</td>
<td>286</td>
<td>ACE, Dg, BB, Du, S, AC</td>
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<tr>
<td>8</td>
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<td>IHD</td>
<td>265</td>
<td>Dob, BB, N</td>
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<td>51</td>
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<td>295</td>
<td>ACE, Du, N, AC</td>
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<td>−6 0 −3.0 2.55</td>
</tr>
<tr>
<td>10</td>
<td>47</td>
<td>IHD</td>
<td>110</td>
<td>ACE, BB, Du, AC</td>
<td>ACE, BB, Du, AC</td>
<td>−10 0 −2.65 −1.32</td>
</tr>
</tbody>
</table>

IHD=Ischemic heart disease; DCM=dilative cardiomyopathy; LVAD=left ventricular end-diastolic diameter assessed by standard echocardiography; MI=mitral insufficiency expressed as score; n.a.=not available.

Dob=dobutamine; ACE=angiotensin-converting enzyme inhibitor; N=nitrate; Dg=digoxin; BB=beta-blocker; Du=diuretic; S=spironolactone; AC=anticoagulation.
per specimen on the nuclear level with the assist of an analysis system (KS 300, Zeiss), we calculated the mean cardiomyocyte diameter as previously described [11]. The interstitial collagen fraction was determined on Sirius-red-stained slides. Twenty visual fields at a final magnification of 200 were analyzed under the polarizing microscope and the total birefractive collagen was detected. Interstitial collagen fractions were calculated as ratios between the collagen area and the total ventricular area in the corresponding section, in percent [11]. Areas with perivascular fibrosis and replacement fibrosis were excluded from the measurement.

2.3. Quantitation of cardiac mRNA expression of natriuretic peptides and receptors by real-time RT-PCR

A quantitative analysis of ANP, BNP, GC-A and NPR-C mRNA expression was performed using the Light-Cycler Detection System (Roche Diagnostics) [12]. The following oligonucleotide primers and fluorogenic probes were used (Tib Molbiol, Berlin): for ANP, sense primer 5'-TTCTCCACCCACCCCGTGA; antisense primer 5'-GGCTCCAATCTGTCCATCC (resulting in a 411-bp amplicon); detection probe 5'-LC-Red 640-CACTGCCCTCGACATTTCA (294-bp amplicon); detection probe 5'-LC-Red 640-CCTGCAGCTCCGACTTCC (resulting in a 411-bp amplicon); anchor probe 5'-GGGCTCCAGGGATGTCTGCTCC; for BNP, sense primer 5'-CACAGCATGAGGTTGACTCTCTGC; anchor probe 5'-ATCGTGGAATCCTTCAACAAACA (542 bp amplicon); detection probe 5'-LC-Red 640-CCTGCAGCTCCGACTTCC (resulting in a 411-bp amplicon); anchor probe 5'-GGGCTCCAGGGATGTCTGCTCC; for GC-A, sense primer 5'-CCAGTTCAAGTCTTTGCCAAG; antisense primer 5'-AACACGCATGCCCTTGTACGA (304 bp amplicon); detection probe 5'-LC-Red 640-CCACCGAGGTGTCCTCTTAAATATGCT; anchor probe 5'-GCCGTTACGGGTCTCAGTTCAC; for NPR-C, sense primer 5'-TCTGGAAACGTCCGGGTTA; antisense primer 5'-LC-Red 640-CCTGCAGCTCCGACTTCC (resulting in a 411-bp amplicon); detection probe 5'-LC-Red 640-CCTGCAGCTCCGACTTCC (resulting in a 411-bp amplicon); anchor probe 5'-ACCACGTGATGCTCAGGATGCT. Quantitative PCR analysis was performed using the Light-Cycler software (Roche Diagnostics) by interpolation with a standard curve generated by using known amounts of the target DNA [13]. All transcripts were determined as described [3]. To initiate cyclase activity, membranes prepared from left ventricular homogenates was the increase in β-receptor blocker recipients (seven patients before, all patients after LVAD) and the reduction of catecholamines (five patients before, one patient after LVAD) (Table 1). Morphometrical analyses of PAS-stained tissue sections demonstrated a significant decrease in cardiomyocyte diameters during LVAD support (pre-LVAD 23.2±2.6 μm, post-LVAD 19.9±2.1 μm; P<0.05). However, cardiomyocytes of CHF patients remained significantly enlarged as compared to nonfailing controls (14.04±2.4 μm) (Table 1). Sirius red stainings revealed increased interstitial collagen fractions that did not change after LVAD support (pre-LVAD 2.7±1.4%, post-LVAD 2.7±0.9%) (Table 1).

3.2. Reversible increases in the expression of ANP, BNP and NPR-C in end-stage heart failure

Quantitative RT-PCR analysis demonstrated that left ventricular mRNA expression levels of ANP (by 39-fold), BNP (by 27-fold) and of the NP-clearing NPR-C receptor
by 4.1-fold) were markedly increased in left ventricular biopsies from CHF patients as compared to nonfailing controls (Fig. 1, pre-LVAD). During chronic hemodynamic unloading, the mRNA levels of ANP and NPR-C markedly decreased almost to the levels in control hearts (Fig. 1, post-LVAD). Similarly, BNP mRNA levels significantly

Fig. 1. Quantitative RT-PCR analyses of ANP, GC-A and NPR-C mRNA in left ventricular biopsies obtained from nonfailing (NF) hearts and from patients with congestive heart failure (CHF) before (pre-LVAD) and after chronic hemodynamic unloading with a left ventricular assist device (post-LVAD). Signal intensities were normalized to β2-microglobulin. Cardiac expression of both ANP and NPR-C is significantly increased in CHF before LVAD implantation (pre-LVAD) as compared to NF controls. GC-A expression is not altered but the ratio of GC-A to NPR-C (GC-A/NPR-C) is decreased in CHF. Comparison of transcript levels in individual LVAD recipients demonstrates that hemodynamic unloading by LVAD (post-LVAD) reverses the changes in ANP and NPR-C expression and increases the ratio of GC-A/NPR-C to the levels observed in NF controls (*P<0.05 vs. NF; *P<0.05 vs. pre-LVAD).

Fig. 2. Scatterplot and regression analysis for cardiac ANP, BNP, NPR-C mRNA levels as well as GC-A/NPR-C ratios, and cardiomyocyte diameters in 10 patients before (closed circles) and following LVAD implantation (open circles). All mRNA levels were normalized to β2-microglobulin as reference gene. Note a significant positive correlation between the ANP, BNP, NPR-C expression levels and cardiomyocyte diameters. A reciprocal trend was observed between GC-A/NPR-C ratios and cardiomyocyte diameters but this did not reach statistical significance.
3.3. Reversible decreases of cardiac GC-A responsiveness to ANP in end-stage heart failure

To further characterize the local activity of the ANP/GC-A system, membrane-bound guanylyl cyclase activity was assayed in left ventricular biopsies from nonfailing control hearts as well as CHF patients before and after LVAD support. ANP-stimulated (10 nM–1 μM) cGMP synthesis in CHF samples was nearly abolished (Fig. 3, pre-LVAD). Reverse remodeling during LVAD support led to a significant increase in ANP-dependent cardiac GC-A activity (Fig. 3). In fact, GC-A responsiveness in “post-LVAD” ventricles was similar to nonfailing controls.

4. Discussion

Our study shows that increased cardiac ventricular ANP and BNP expression in CHF patients is accompanied by increased cardiac expression of the NP-metabolizing NPR-C receptor, decreased ratios of GC-A to NPR-C receptors, and abolished cGMP-responsiveness of GC-A to ANP. Reverse remodeling of the heart during chronic hemodynamic unloading by LVAD reverses these changes and reintegrates the local responsiveness of GC-A to ANP. Our recent observations in mice with cardiomyocyte-restricted deletion of GC-A [3] suggest that recuperation of the protective cardiac effects of ANP and BNP might contribute to the partial reversal of cardiac hypertrophy observed in CHF patients during LVAD support.

Several studies have shown that GC-A mRNA and receptor number are reduced in cultured cells stimulated for a prolonged period of time with high ANP concentrations [15]. In fact, functional analysis of the GC-A gene promoter revealed a putative cGMP-responsive element, suggesting that the ANP-dependent down-regulation of GC-A mRNA is cGMP dependent [16,17]. However, other studies could not reproduce this result [18]. In accordance with the latter, in the present study, cardiac GC-A mRNA expression levels were not affected by the marked increases (pre-LVAD) and decreases (post-LVAD) in ANP and BNP levels. Thus, it is possible that different types of tissues and cells rely on different mechanisms to regulate GC-A expression. In addition, the referred studies [16,17] were performed in tissue culture and cells of nonhuman origin and the processes modulating GC-A expression under these in vitro conditions may differ markedly from the processes regulating GC-A expression in the human myocardium in vivo.

In line with our study, published reports have already suggested that attenuation of the endocrine vasodilating and diuretic/natriuretic responses of CHF patients to ANP and BNP is partly due to an up-regulation of NPR-C receptors, leading to increased sequestration and degradation of these peptides [9,10]. Even more, in our study, cardiac ANP/BNP and NPR-C expression levels were positively correlated. Increased NPR-C expression probably contributes to but not fully explains the blunted cGMP response of systemic [8] and cardiac GC-A to NPs. Biochemical modifications of GC-A such as dephosphorylation in the presence of high ANP/BNP concentrations (homologous desensitization) or of growth factors such as Angiotensin II and endothelin (heterologous desensitization) might also be involved (comprehensively reviewed in Ref. [19]). Although these processes so far have only been demonstrated in vitro, they may also interfere with NP/GC-A signaling under certain pathological conditions in vivo, particularly in diseased hearts. Ultimately, which of these processes (increased NPR-C expression, posttranslational modifications of GC-A) account for the diminished responsiveness of cardiac GC-A in CHF patients remains an open and important question for our future studies.
Morphometrical analyses in comparison with nonfailing control hearts showed that hemodynamic unloading significantly but not fully reversed cardiac hypertrophy in CHF patients. Notably, overall, there was a clear positive correlation between cardiomyocyte diameters and cardiac ANP, BNP as well as NPR-C mRNA expression levels. However, whereas cardiac hypertrophy did not fully reverse during LVAD support, the changes of ANP, BNP and receptors were completely reversible (i.e., cardiac NP and NPR-C expression levels as well as GC-A activity “post-LVAD” were not significantly different from nonfailing controls), suggesting that they are partly independent from cardiac hypertrophy but also dependent from other local variables such as myocyte stretch. In addition, patient medication might also have a role. For instance, β-adrenoceptor antagonists are known to augment plasma ANP, BNP and cGMP concentrations despite their hypotensive actions [20]. However, in our study, group β-blocker therapy was intensified during LVAD support and despite this, ANP and BNP expression levels markedly decreased. It was also reported that β-blockers can decrease NPR-C expression levels and thereby increase cGMP-responsiveness to ANP [21]. Thus, the increase in β-blocker medication and the reduction of catecholamines (see Table 1) might contribute to diminished NPR-C expression and recuperation of GC-A/cGMP responsiveness after LVAD support.

Intriguingly, the regression of cardiac hypertrophy during LVAD support was not accompanied by a significant reduction of total interstitial collagen content, as quantified on Sirius-red-stained tissue slides. These results are consistent with a previous report by Li et al. [22], which, by a biochemical method, also demonstrated that total cardiac collagen content does not change after LVAD support, the duration of support being similar to our study. However, by biochemical analyses, these authors demonstrated that the ratio of insoluble (undenatured) to total collagen increases during hemodynamic unloading, together with a down-regulation of matrix metalloproteinases (MMP) [22]. Thus, reduced MMP activity may lead to diminished damage to the matrix collagen, and, together with the regression of cardiomyocyte hypertrophy observed in the present as well as other published studies [23], contribute to the functional recovery and LV plasticity after LVAD support.

5. Limitations

Because of the low patient number, this study is not able to differentiate between dilated cardiomyopathy and ischemic heart disease. Another important limitation is the lack of specific antibodies for immunohistochemical studies, which hindered us to distinguish which of the various cell types within the heart account for the changes in NPR-C expression levels. Third, this study could not address the functional implications of the increased cardiac NPR-C expression levels in CHF patients, i.e., whether these changes in receptor expression were accompanied by increased local internalization and degradation of ANP and BNP. Last, our study could not identify the posttranslational modifications of cardiac GC-A possibly contributing to the diminished responsiveness to ANP in CHF. The development of phosphospecific antibodies to characterize GC-A dephosphorylation/desensitization [19] in vivo will be a main goal for our future studies.

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