Deloading of the Left Ventricle by Ventricular Assist Device Normalizes Increased Expression of Endothelin ET<sub>A</sub> Receptors But Not Endothelin-Converting Enzyme-1 in Patients With End-Stage Heart Failure

Henning Morawietz, PhD; Marten Szibor, MD; Winfried Goettsch, MS; Babett Bartling, MS; Matthias Barton, MD; Sidney Shaw, PhD; Reiner Koerfer, MD; Hans-Reinhard Zerkowski, MD; Juergen Holtz, MD

Background—Ventricular assist devices (VAD) are implanted in patients with end-stage heart failure for bridging the time until heart transplantation, resulting in hemodynamic unloading of the failing heart, improved cardiac contractile and mitochondrial function, and reversal of cardiac hypertrophy. It is unknown whether VAD unloading may affect the cardiac endothelin (ET) system, which has been proposed as one of the putative pathomechanisms of heart failure.

Methods and Results—With the use of standard-calibrated, competitive reverse-transcription–polymerase chain reaction mRNA expression of components of the ET system was analyzed in left ventricular myocardium from nonfailing donor hearts, from failing hearts without and with ACE inhibitor therapy, and from patients with end-stage heart failure at the time of VAD implantation and 103±15 days after VAD implantation during removal with subsequent heart transplantation. ET receptor A (ET<sub>A</sub>) was markedly upregulated in failing human myocardium. This increased ET<sub>A</sub> expression was not affected by ACE inhibitor treatment but was normalized by VAD unloading. ET<sub>A</sub> expression before or after VAD implantation did not correlate with duration of VAD implantation or suppression of Pro-ANP mRNA. ET<sub>B</sub> mRNA expression was unaffected by heart failure or VAD. In contrast, increased ET-converting enzyme-1 mRNA and ET-1 peptide levels in failing myocardium were partially normalized by ACE inhibition but not by VAD unloading.

Conclusions—We conclude that VAD implantation normalizes ET<sub>A</sub> expression in failing human left ventricular myocardium, probably as the result of the beneficial effects of VAD unloading. (Circulation. 2000;102[Suppl III]:III-188-III-193.)

Key Words: endothelin ■ heart failure ■ heart-assist device
rates in human heart failure. Furthermore, in an experimental infarct model of heart failure, chronic ET-receptor antagonism was associated with a reduction in ventricular remodeling and mortality rates. In human myocardium, both ETA and ETB are expressed, but only ETB has been shown to be of functional importance. Recently, upregulation of ETB receptor-binding sites has been demonstrated in failing human myocardium of patients with dilated cardiomyopathy (DCM).

The expression of ECE-1 in failing human ventricular myocardium has been analyzed in less detail. In a recent study, no differences in ECE-1 expression could be detected in hearts of nonfailing donors, compared with patients with DCM. However, the impact of therapy by inhibitors of ACE, the gold standard in the treatment of heart failure, on the expression of genes of the ET system in failing human left ventricular myocardium is currently unknown. Angiotensin II and ET-1 act synergistically in the development of cardiac hypertrophy. In addition, ET-1 and Angiotensin II have been shown to mediate hypertrophic response in cultured cardiomyocytes exposed to mechanical stretch in vitro.

Therefore, we investigated the effect of hemodynamic unloading by the VAD on the expression of components of the human ET system in myocardium of patients with end-stage heart failure. In these studies, we obtained left ventricular specimens from the same patients with heart failure during VAD implantation (maximal hemodynamic overload) and afterward during heart transplantation with VAD removal (substantial temporal hemodynamic unloading by the VAD). In addition, we analyzed left ventricular myocardium of patients with and without ACE inhibitor therapy (without previous VAD support before heart transplantation) for comparison. In these human ventricular specimens, we analyzed the impact of hemodynamic unloading and of ACE inhibitor therapy on the expression of ETB, ECE-1, and ET-1 in failing human myocardium.

Methods

Patient Population
Left ventricular specimens were obtained after informed consent from 10 male patients (8 with DCM; 2 with ischemic cardiomyopathy, ICM) undergoing VAD implantation as a bridge to transplantation at the time of surgery and at removal of the VAD with subsequent orthotopic heart transplantation. At the time of VAD implantation, patients were 53 ± 4 years of age. The hemodynamic data were obtained from the date where the last echocardiographic determination of the ejection fraction (24 ± 1%) before VAD implantation was performed. For left ventricular unloading, either TCI HeartMate (Thermo Cardiosystems, Inc, n = 3), Novacor (Baxter Healthcare Corp, n = 6), or Thoratec (Thoratec Laboratories, Inc, n = 1) assist devices were used. For biventricular support in the case of TCI or Novacor use, an additional right ventricular assist device of Thoratec was implanted (n = 2). The average time on VAD was 103 ± 15 days (range 36 to 169 days). Cardiac index was improved by hemodynamic unloading from 1.9 ± 0.2 L min⁻¹ m⁻² before VAD implantation (before VAD; n = 10) to 2.9 ± 0.3 L min⁻¹ m⁻² after VAD implantation (after VAD in those patients in whom measurements were possible immediately before transplantation; n = 4) (P < 0.05 versus before VAD). Pulmonary capillary wedge pressure was reduced by VAD from 29 ± 2 mm Hg before VAD to 14 ± 3 mm Hg after VAD (P < 0.05 versus before VAD). Mean aortic pressure was increased by VAD support from 69 ± 3 mm Hg before VAD to 78 ± 3 mm Hg after VAD, without reaching statistical significance (P = 0.058 versus before VAD).

In addition, explanted left ventricular specimens of patients transplanted by the Halle and Hamburg Cardiac Transplant Program (6 men, 56 ± 2 years old) with end-stage heart failure (ICM) and treated with β-blockers (n = 1), nitric oxide donors (n = 1), calcium antagonists (n = 2), and diuretics (n = 5) but not with ACE inhibitors or VAD unloading were investigated, with an average ejection fraction of 22 ± 4%. Finally, 11 patients (9 men, 2 women) with end-stage heart failure (8 DCM, 3 ICM; 53 ± 3 years old) treated with ACE inhibitors (n = 11), β-blockers (n = 1), nitric oxide donors (n = 2), calcium antagonists (n = 2), and diuretics (n = 5) but not with VAD unloading were studied. Long-term ACE inhibitor treatment before surgery was assessed in a retrospective manner. The ACE inhibitor dosages prescribed by the referring physicians were 31 ± 25% of respective target dosages in recent heart failure mega-trials. The mean ejection fraction of this group with ACE inhibition was 28 ± 3%. Left ventricular tissues from 7 organ donors without structural abnormalities (4 men, 3 women; 35 ± 6 years old) served as controls. These hearts were not transplanted for technical reasons. The local ethics committee approved the study of these human cardiac tissues.

RNA Preparation
Left ventricular specimens of patients were dissected immediately after explantation, snap-frozen, and stored in liquid nitrogen until RNA preparation. Total RNA from myocardial tissue was isolated by guanidinium thiocyanate/cesium chloride centrifugation as described. The RNA was analyzed by standard agarose gel electrophoresis with ethidium bromide, and RNA concentration was determined by UV spectrophotometry.

Standard-Calibrated, Competitive Reverse Transcriptase–Polymerase Chain Reaction
Human ECE-1, ETB, and Pro-ANP mRNA was quantified by standard-calibrated reverse transcriptase–polymerase chain reaction (RT-PCR). For human ECE-1 mRNA, a new standard-calibrated, competitive RT-PCR was established. In brief, a human ECE-1-specific cDNA fragment of 215 bp was amplified by PCR with the use of the following primers: ECE-1 sense primer: 5′-GAA GCG GCT GGT GGT GTT GGT G3′ and ECE-1 antisense primer: 5′-GTT TCG CTA TTC AGC-3′, respectively. The ECE-1-specific cDNA fragment was subsequently cloned into the pCR II–TOPO vector (Invitrogen), and its identity was confirmed by DNA sequencing (ABI PRISM® Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS, Perkin-Elmer Co; ABI 373 DNA Sequencer). Second, an internal deleted ECE-1 cDNA standard (133 bp) was constructed by modification of a linker primer method. The identity of the standard was confirmed by cloning and DNA sequencing. Third, internal deleted cDNA standard was transcribed in vitro into cRNA (RNA Transcription Kit, Stratagen). Standard cRNA was quantified spectrophotometrically.

In competitive RT-PCR experiments, equal amounts of total RNA from human myocardium (200 ng) were incubated in separate reactions with defined amounts of serial 1:3 dilutions of ECE-1 standard cRNA for 3 minutes at 70°C and subsequently reverse transcribed into cDNA with random hexamer primers and SuperScript II RNase H reverse transcriptase (Life Technologies) for 1 hour at 42°C. Afterward, 20% of each reverse transcription reaction was amplified in separate reactions with 20 pmol/L ECE-1 sense and antisense primers by the following PCR protocol: 30 seconds 95°C, 30 seconds 62°C, 30 seconds 72°C (36 cycles). PCR primers compete for sample-specific and standard molecules in the amplification reaction. The PCR reactions were separated by standard agarose gel electrophoresis, stained with ethidium bromide, and documented by photography with Polaroid film type 665. The optical density of standard and sample-specific PCR fragment was estimated by a Personal Densitometer (Molecular Dynamics). Optical density of standard PCR fragments was normalized with a correction coefficient (ECE-1: 215 bp/133 bp = 1.62) and logarithm of the
quotient of normalized standard and sample-specific PCR fragment density was graphically plotted versus amount of standard RNA molecules, with the SigmaPlot scientific graphing software (Jandel Corp). In the graph, equal amounts of RNA molecules in sample and standard were present at equivalence point.

The mRNA expression of human ETₐ, ETₐ, and Pro-ANP was quantified by standard-calibrated competitive RT-PCR as described previously. The characteristics of quantitative mRNA analyses in human left ventricular myocardium by competitive RT-PCR (PCR protocol: 30 seconds at 95°C, 30 seconds at primer-specific annealing temperature, and 30 seconds at 72°C) are summarized in the Table.

**Tissue Endothelin Protein Content**

Total protein from left ventricular tissues was isolated as described. Protein concentration was determined by the Bio-Rad protein assay. Tissue ET-1 content was determined with isoform-specific rabbit antibodies against synthetic ET-1 (Peninsula Laboratories) and reverse-phase high-performance liquid chromatography as described previously.

**Statistics**

Data are given as mean±SEM. The ANOVA procedure followed by Bonferroni’s method (multiple comparison) or Student’s t test was used for statistical comparison as appropriate (SigmaStat software, Jandel Corp). The correlation coefficient r of the linear regression analysis for significance was tested by a 2-sided test. A value of P<0.05 was considered statistically significant.

**Results**

**Normalization of Increased ETₐ Expression by VAD in Failing Human Myocardium**

The mRNA expression of genes of the human endothelin system was determined by standard-calibrated, competitive RT-PCR (in amol/µg RNA) in RNA from left ventricular myocardium (Figure 1). Myocardial ETₐ expression was upregulated in patients with heart failure (20±2 versus 12±1 amol/µg RNA in donor hearts, P<0.05) (Figure 2A). This augmented ETₐ expression in failing human myocardium was not affected by concomitant ACE inhibitor therapy (20±2 without versus 19±3 amol/µg RNA with ACE inhibition). Hemodynamic unloading by VAD normalized increased ETₐ expression in the myocardium of patients with end-stage heart failure and ACE inhibitor therapy before implantation of the VAD (before VAD: 18±2 amol/µg RNA) compared with myocardium from the same patient after transplantation and VAD removal (after VAD: 12±1 amol/µg RNA, P<0.05 versus before VAD) (Figure 2B). ETₐ expression was reduced in 7 of 10 patients, whereas the remaining 3 patients with initially lower ETₐ levels showed no further decrease of expression.

However, although we observed reduced left ventricular Pro-ANP expression after VAD-induced hemodynamic unloading (before VAD: 180±66 versus after VAD: 28±9 amol/µg RNA, P<0.05; donor hearts: 22±12 amol/µg RNA) and although a role of ETₐ in volume load-induced cardiac ANP release has been described, no correlation of reduced ETₐ and Pro-ANP expression by VAD was found. Furthermore, duration of VAD implantation did not correlate with ETₐ expression (data not shown).

**Myocardial ET₉ Expression**

No significant changes of left ventricular ET₉ receptor expression could be found in failing hearts from patients with or without ACE inhibitor therapy (donors: 17±2, explanted without ACE inhibitor: 22±4, explanted with ACE inhibitor: 23±4 amol/µg RNA; P=0.5, NS) (Figure 3A). Furthermore, hemodynamic unloading by VAD had no effect on left ventricular ET₉ expression (before VAD: 17±2 versus after VAD: 20±4 amol/µg RNA, P=0.45, NS) (Figure 3B).
Upregulation of ECE-1 in Failing Human Myocardium

In left ventricular myocardium of patients with heart failure and medical treatment without ACE inhibitors, determined in a retrospective manner, we found a significant upregulation of ECE-1 expression (58±4 versus 34±7 amol/μg RNA in donor hearts, P<0.05) (Figure 4A). This increased ECE-1 expression in failing hearts was partially reduced by ACE inhibitor therapy (48±2 amol/μg RNA). A similar regulation was seen in tissue ET-1 peptide content in myocardium from patients with end-stage heart failure before VAD implantation (pre VAD, white bar) and after VAD support (post VAD, dashed bar). ECE-1 mRNA is upregulated in left ventricle of patients with heart failure (A). This increased expression can be normalized by hemodynamic unloading with VAD (B). Data are expressed as mean±SEM (amol/μg RNA).

Discussion

This study for the first time demonstrates normalization of increased ET_A receptor expression in failing left ventricular myocardium of patients by hemodynamic unloading with VAD support. Our data of increased ET_A expression in failing left ventricular myocardium are in agreement with recent reports describing an upregulation of ET_A-specific binding sites in myocardium of patients with heart failure. In human left ventricular myocardium, only ET_A receptors have been shown to be of functional importance. These data not only support a role for the cardiac ET system in heart failure but suggest furthermore that mechanical left ventricular off-loading by VAD downregulates the augmented expression of components of the ET system in failing human hearts in vivo. ET-1 stimulates hypertrophy, contractility, protein synthesis, and phosphoinositide hydrolysis in cardiac myocytes by ET_A receptors. In experimental studies, chronic endothelin receptor blockade improved cardiac function, attenuated progressive ventricular dilation, and reduced mortality rates in an infarct model of heart failure. Recently, chronic ET_A receptor blockade was shown to attenuate left ventricular hypertrophy independent of blood pressure effects in hypertensive rats. The augmented ET_A expression found in failing human myocardium may provide the target of a new therapeutic strategy in the treatment of heart failure by ET_A receptor antagonism. It is important to note that in the patients in our study, no effect of ACE inhibition, the standard therapy in the treatment of heart failure, on ET_A expression in left ventricular myocardium of patients with heart failure was observed.

Because of the limitations in tissue availability, in this study only ET_A expression studies on mRNA level could be performed. We cannot determine whether in left ventricular homogenates ET_A upregulation was due to expression in cardiac myocytes or nonmyocytes (eg, endothelial cells, smooth muscle cells, or fibroblasts). However, we and others...
In addition, an increased ET\textsubscript{A} receptor density in failing fibroblasts does not exceed ET receptor number on myocytes. Increased ET\textsubscript{A} mRNA levels most likely reflect increased ET\textsubscript{A} receptor protein expression and function on myocytes of patients with heart failure. Our tissue peptide measurements indicate an increase of local ET synthesis in failing human myocardium of patients with ACE inhibitor therapy. These data suggest an additional beneficial effect of ACE inhibitor therapy. However, ECE-1 expression was still slightly elevated in left ventricular myocardium of patients with ACE inhibitor therapy. These data suggest an additional beneficial effect of ACE inhibitor therapy. This result further supports the importance of the observed normalization of increased ET\textsubscript{A} receptor expression by the VAD.

The upregulation of ECE-1 expression in left ventricular myocardium of patients with heart failure suggests a novel therapeutic strategy in the treatment of heart failure by ECE inhibition. A similar upregulation of ECE-1 has been found in failing human atrial myocardium (Morawietz, unpublished data, 1999). A local increased ECE-1 expression in failing human myocardium could promote increased local ET synthesis in cardiomyocytes and in increased local proteolytic cleavage of systemically elevated big ET-1 levels in patients with heart failure. Our tissue peptide measurements indicate an increase of local ET synthesis in failing human myocardium. Interestingly, we found a partial normalization of elevated ECE-1 expression in failing human myocardium of patients with ACE inhibitor therapy. These data suggest an additional beneficial effect of ACE inhibitor therapy. However, ECE-1 expression was still slightly elevated in left ventricular myocardium of patients with ACE inhibitor therapy, suggesting a putative beneficial effect of ECE inhibition in the treatment of heart failure. The effect of ACE inhibitor therapy might explain the differences of our data, with a recent study showing no significant change in ECE-1 expression in failing human left ventricular myocardium. The myocardial ECE-1 expression was not changed during VAD unloading. Therefore, reduced ET\textsubscript{B} expression is not counterbalanced by altered ECE-1 expression in myocardium of patients with heart failure and without ACE inhibitor therapy.
patients under VAD support, further supporting a beneficial effect of normalization of ET<sub>A</sub> receptor levels.

In conclusion, hemodynamic unloading by the VAD normalized ET<sub>A</sub> receptor expression in the left ventricular myocardium of patients with end-stage heart failure. This effect might contribute to the beneficial effects of the VAD on phenotypic and functional changes in failing human myocardium. Furthermore, we found an interaction of the human renin-angiotensin and the ET system on the level of myocardial ECE-1 expression. Pharmacological therapy of locally elevated ET<sub>A</sub> and ECE-1 expression by ET<sub>A</sub>-specific receptor blockade or ECE inhibition might provide new therapeutic perspectives in the treatment of heart failure.

**Acknowledgments**

This study was supported by the Novartis foundation for therapeutic research (Dr Morawietz), grants of the German Bundesministerium für Bildung und Forschung (BMBF, 01ZZ9512-TV3, and TV7) (Drs Holtz and Zerkowski), the Deutsche Forschungsgemeinschaft (Ba/543/1-1), and the Swiss Heart Foundation (Dr Barton). We are grateful to R. Gall, B. Heinze, and R. Busath for excellent technical assistance. The authors are indebted to J. Boden for her help with the radioimmunoassays. Furthermore, we deeply express our appreciation for the cooperation of the patients and staff of the cardiothoracic surgery clinics at the Heart Center in Bad Oeynhausen and at the Martin Luther University in Halle, Germany.

**References**

DeKing the Left Ventricle by Ventricular Assist Device Normalizes Increased Expression of Endothelin ET\textsubscript{A} Receptors But Not Endothelin-Converting Enzyme-1 in Patients With End-Stage Heart Failure
Henning Morawietz, Marten Szibor, Winfried Goettsch, Babett Bartling, Matthias Barton, Sidney Shaw, Reiner Koerfer, Hans-Reinhard Zerkowski and Juergen Holtz

_Circulation_. 2000;102:Iii-188-Iii-193
doi: 10.1161/01.CIR.102.suppl_3.III-188

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/102/suppl_3/Iii-188

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/