

Myocardial Gene Expression of Regulators of Myocyte Apoptosis and Myocyte Calcium Homeostasis During Hemodynamic Unloading by Ventricular Assist Devices in Patients With End-Stage Heart Failure

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Background—In patients with end-stage heart failure, characterized by an increased susceptibility to cardiomyocyte apoptosis and a labile cardiomyocyte calcium homeostasis, a ventricular assist device (VAD) is implanted for bridging to cardiac transplantation and results in myocardial unloading. Although phenotype changes in the failing heart are assumed to result from hemodynamic overload, the reversibility of these changes under unloading is unknown.

Methods and Results—By use of quantitative reverse-transcription polymerase chain reaction, mRNA expression analyses were performed on left ventricular specimens obtained from 10 nonfailing donor hearts (from 8 patients with dilated cardiomyopathy and 2 patients with coronary heart disease) at the time of VAD implantation and 36 to 169 days later during VAD removal with subsequent cardiac transplantation. In terminally failing hearts before VAD support, left ventricular mRNA analyses revealed increased Pro-ANP, reduced antiapoptotic Bcl-x_L and antiapoptotic Fas isoform FasExo6Del, and a decreased ratio of sarcoplasmic reticulum Ca²⁺-ATPase per sarcolemmal Na⁺-Ca²⁺ exchanger in comparison with nonfailing ventricles. After VAD unloading, ventricular transcription of Pro-ANP was immediately normalized, and apoptotic DNA fragmentation was attenuated. In patients with dilated cardiomyopathy, mRNAs of Bcl-x_L and FasExo6Del/Fas were enhanced depending on time on VAD. The Bcl-x_L mRNA level correlated positively with that of the Bcl-x_L protein. Transcription of sarcoplasmic reticulum Ca²⁺-ATPase/Na⁺-Ca²⁺ exchanger demonstrated recovery in only 4 of 10 patients.

Conclusions—Mechanical support of the failing heart induces a time-dependent change in myocardial gene expression compatible with a decreased susceptibility to apoptosis. (*Circulation*. 1999;100[suppl II]:II-216–II-223.)

Key Words: apoptosis ■ calcium ■ heart failure ■ heart-assist device

Currently, ventricular assist devices (VADs) are implanted for mechanical support of a failing human heart and function reliably for weeks or months without complications even outside the hospital.¹ A shortage of organ donors for heart transplantation has led to the increased application of VADs for patients in end-stage heart failure (HF); the VAD serves as a bridge to transplantation. This bridging also improves the outcome after the subsequent heart transplantation.^{2,3}

The overload-induced distension of the heart is a critical factor promoting the progression of HF.⁴ Therefore, it has been postulated that some myocardial recovery should occur in the failing heart hemodynamically unloaded by an implanted VAD, and several clinical reports indicate some ventricular recovery under VAD support.^{5,6}

Experimental investigations revealed that mechanical distension of the myocardium induces apoptotic death of cardiomyocytes.⁷ Furthermore, apoptosis is demonstrable in ventricles of patients with a terminally failing, overloaded heart,^{8,9} which is associated with mRNA downregulation of antiapoptotic regulators, such as Bcl-x_L¹⁰ and FasExo6Del.¹¹ In human myocardium, FasExo6Del is the most abundant¹¹ of several soluble antagonistic isoforms of the apoptosis-triggering surface receptor Fas,¹² and it is assumed to function by competing with Fas for the Fas ligand. In contrast, the antiapoptotic Bcl-2 family member Bcl-x_L is an intracellular protein localized at the outer mitochondrial membrane.¹³ It prevents the apoptosis-associated disruption of the mitochondrial transmembrane potential,¹⁴ the release of cytochrome *c* from the mitochondrial intermembrane space, and the cyto-

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TABLE 1. Clinical Data for 10 Male Patients Before Ventricular Support by the Assist Device and Thereafter, Shortly Before Orthotopic Heart Transplantation

Patient No.	Age, y	HF	Ejection Fraction, %	Drug Treatment		Time on VAD, d	Cardiac Index, L · min ⁻¹ · m ⁻²		Pulmonary Capillary Wedge Pressure, mm Hg		Mean Aortic Pressure, mm Hg		Cardiac DNA Fragmentation, RU	
				Pre-VAD	VAD		Pre-VAD	Pre-HTx	Pre-VAD	Pre-HTx	Pre-VAD	Pre-HTx	Pre-VAD	Pre-HTx
1	62	DCM	26	ACE i	Novacor LVAD	36	1.33	3.40	25	11	67	75	ND	ND
2	42	DCM	24	ACE i	TCI LVAD	42	1.75	ND	25	ND	69	85	0.42	0.41
3	63	DCM*	25	ACE i	Novacor LVAD	57	2.08	ND	16	10	59	ND	0.58	0.27
4	45	DCM	28	ACE i	Novacor LVAD	64	2.20	2.16	28	4	62	79	0.36	0.21
5	47	DCM	26	ACE i	TCI LVAD	116	1.53	ND	41	9	87	85	0.42	0.31
6	49	DCM	25	ACE i	Novacor/Thoratec L/RVAD	117	2.79	3.16	34	ND	67	63	0.69	0.66
7	59	DCM†	18	ACE i	Novacor LVAD	140	1.46	ND	36	ND	69	ND	0.58	0.14
8	30	DCM	25	ACE i	Novacor LVAD	169	1.36	ND	30	22	78	ND	0.43	0.63
9	62	CAD	28	AT-1 bl	Thoratec/Thoratec L/RVAD	143	1.90	ND	30	25	70	ND	0.23	0.04
10	68	CAD	15	...	TCI LVAD	144	2.69	2.80	27	17	57	79	0.53	0.37
Mean±SD	53±12		24±4			103±49	1.9±0.5	2.9±0.5	29±7	14±8§	69±8	77±8	0.5±0.1	0.3±0.2‡

HTx indicates heart transplantation; RU, relative units (integral area of the 200-bp DNA fragment/μg DNA); ACE i, ACE inhibitor; AT-1 bl, angiotensin-1 receptor blocker; ND, not determined; and L/RVAD, left/right VAD.

*With hypertensive heart disease.

†With diabetes mellitus II.

‡ $P < 0.07$, § $P < 0.05$ vs Pre-VAD.

chrome *c*-mediated activation of the apoptotic process.¹⁵ Heterodimerization with the antiapoptotic Bcl-2-related protein Bak through homology domains, characteristic for members of the Bcl-2 family, inhibits the effect of Bcl-x_L.¹³ Like Bcl-x_L, Bcl-2 and Mcl-1 exert an analogous apoptosis-preventing function, whereas their action is antagonized by Bax.^{16,17}

Presently, the clinical importance of myocardial recovery under VADs remains unclear. Therefore, we investigated whether a reduction of this distension by VAD unloading^{6,18,19} abolishes the fatal trend toward ongoing myocardial apoptosis with normalization of the apoptosis-susceptible myocardial phenotype. Accordingly, we obtained left ventricular specimens from the same heart-failure patients during VAD implantation (ie, in the state of maximal hemodynamic overload) and later, during the transplantation with VAD removal (ie, in the state of substantial temporal unloading by the VAD). The left ventricles of HF patients who were successfully transplanted but without previous VAD support were additionally investigated for comparison.

Apart from the apoptosis-sensitive myocardial phenotype, the left ventricular expression of proteins involved in the myocyte calcium homeostasis is altered in failing human myocardium. In detail, a decreased expression of the sarcoplasmic reticulum (SR) Ca²⁺-ATPase²⁰ is associated with an enhanced expression of the sarcolemmal Na⁺-Ca²⁺ exchange-

er²¹ in end-stage HF. Therefore, we additionally analyzed the left ventricular mRNA levels of both calcium-regulatory determinants under VAD support of the overloaded heart.

Methods

Patient Population

We obtained left ventricular specimens from 10 male patients undergoing VAD implantation as a bridge to transplantation at the time of surgery and at removal of the VAD with subsequent orthotopic cardiac transplantation. Eight patients suffered from dilated cardiomyopathy (DCM), and 2 patients demonstrated end-stage coronary artery disease (CAD). At the time of VAD implantation, patients were 53±12 years old and clinically characterized as shown in Table 1. The hemodynamic data in Table 1 result from the date when the last echocardiographic determination of the ejection fraction was performed. For left ventricular unloading, either TCI HeartMate (Thermo Cardiosystems, Inc), Novacor (Baxter Healthcare Corp, Novacor Division), or Thoratec (Thoratec Laboratories, Inc) assist devices were used. For biventricular support in case of TCI or Novacor use, an additional right ventricular assist device of Thoratec was implanted.

In addition, explanted left ventricular specimens of 22 male and 2 female patients (54±8 years old) from the Halle and Hamburg Cardiac Transplant Program exhibiting end-stage HF and treated without VAD unloading were investigated. They had a mean ejection fraction of 25±15%. Thirteen patients suffered from DCM; 11 patients, from CAD. Left ventricular tissues from 10 organ donors (8 men and 2 women, 39±5 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.

TABLE 2. Characteristics of Quantitative mRNA Analyses of Several Genes in the Human Left Ventricular Myocardium

	Primer Orientation	Human 5'-3' Primer Sequence	Primer Annealing, °C	RNA per RT, ng	cRNA Standard Dilutions, amol	PCR Cycles
					1-2-3-4	
Bak	Sense	CTGTTTTTACCGCCATCAGCAGG	60	250	0.64-0.32-0.11-0.064	33
	Antisense	CTCTCAAACAGGCTGGTGGCAATC				
Bax	Sense	ACCCGAGCTGGCCCTGGAC	60	250	4.04-2.02-0.41-0.20	33
	Antisense	ATGGTGAGCGAGGCGGTGAG				
Bcl-2	Sense	GACTTCTTCGCCGCTACCG	50	250	0.14-0.06-0.03-0.014	32
	Antisense	GACAGCCAGGAGAAATGAAAC				
Bcl-x _L	Sense	GGTGGTTGACTTTCTCTCCTAC	58	250	222-111-44.4-22.2	32
	Antisense	GAAAAGTATCCAGCCGCC				
Fas and FasExo6Del	Sense	GGACCCAGAATACCAAGTG	56	250	3.3-1.0-0.3-0.1	38
	Antisense	CTGTTCTGCTGTGTCTTGG				
Mcl-1	Sense	CGCCATCGTGTCTCCGGAG	60	250	899-450-300-225	33
	Antisense	AAGGCCGTCTCGTGGTTGC				
Na ⁺ -Ca ²⁺ exchanger	Sense	ACCATCGAAGGGACTGCCAGAG	63	30	8000-4000-800-400	30
	Antisense	CTGCAATGCGCCTCTCCTCTTC				
p150.95	Sense	ATTCAGGCGCACGTCAAACC	62	250	*	32
	Antisense	TGAAGCCCTCCTGTGCCATC				
Pro-ANP	Sense	GCATTCCAGCTCCTAGGTGAGA	60	60	3860-1900-390-190	31
	Antisense	GGCTCCAATCCTGTCCATCC				
SR Ca ²⁺ -ATPase 2A/B	Sense	CGGACTTTGAAGGCGTGGATTG	60	30	226-112-56-28 [10 ³]	30
	Antisense	AGGTCCAGGTAGTTGCGGG				

*Semi-quantitative RT-PCR without cRNA standard.

DNA Preparation and Gel Electrophoresis

Genomic DNA was prepared from human left ventricular specimens by use of the Puregene DNA Isolation Kit (Biozym). DNA (1.5 μg) was electrophoretically separated in a SYBR Green-stained agarose gel (Biozym). Apoptotic DNA fragmentation was determined by scanning with a laser densitometer that incorporated an evaluation system (Molecular Dynamics).

TUNEL Assay

Terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL)-positive cardiomyocytes were detected in left ventricular cryosections by using the protocol described in the Apoptaq Peroxidase Kit (Oncor). Positive and negative control sections were included. Microscopic evaluations were performed by using an IMT-2 inverted research microscope (Olympus).

Construction of cRNA Standards and Competitive Reverse-Transcription Polymerase Chain Reaction

The standard cRNA for each human gene under study was constructed by introducing a definite deletion of ≈100 bp into the respective cDNA target molecules of the polymerase chain reaction (PCR) amplification. Each standard cDNA was cloned into the pCR-Script SK 197 plasmid (Stratagene) and in vitro-transcribed from the flanking T3 or T7 promotor to yield cRNA.

For competitive reverse-transcription PCR (RT-PCR), a dilution series of the respective cRNA standard molecules was added to 4 RT reactions containing the same amount of total RNA (Table 2) and simultaneously reverse-transcribed by using SuperScript Plus reverse transcriptase (GIBCO-BRL). Total RNA was isolated from left ventricular specimens after mechanical pulverization in liquid nitrogen using the protocol of Chirgwin et al.²² The RNA concentration was calculated from the absorption at 260 nm.

Five microliters of the first-strand cDNA reaction (RT) was used as the template for PCR, with each containing the following components: 10 pmol of each primer, 1× complete buffer,

12 μmol/L of each dNTP, and 2 U of *rTaq* DNA polymerase (Pharmacia) in a final volume of 50 μL. After an initial denaturation at 95°C for 2 minutes, PCR cycles were performed in a thermocycler (Biometra Triblock) corresponding to the following protocol: 30 seconds at 94°C, 30 seconds at the primer-specific annealing temperature (Table 2), and 30 seconds at 72°C. After extension of the cDNA amplification at 72°C for 5 minutes, the PCR products were electrophoretically separated in an agarose gel containing ethidium bromide (Sigma-Aldrich) for detection. The RT-PCR was evaluated by scanning with a laser densitometer and computer-based imaging system (Molecular Dynamics).

The nucleotide sequences of cDNA clones and PCR fragments were identified by cycle sequencing using the ABI Prism Dye Terminator Cycle Sequencing Kit (Perkin-Elmer) and subsequent automated analysis (Perkin-Elmer, Applied Biosystems Division).

Western Blot

After mechanical pulverization in liquid nitrogen, total protein from left ventricular tissues was extracted in lysis buffer (10 mmol/L Tris-HCl [pH 7.4], 1% SDS, and complete protease inhibitor [Boehringer]) by homogenization and subsequent incubation at 95°C for 15 minutes. Protein concentration was measured by the Bio-Rad protein assay, and samples containing 80 μg of total protein were mixed with 2× loading buffer (250 mmol/L Tris-HCl [pH 7.4], 20% glycerol, 4% SDS, 40 mmol/L dithiothreitol, 2 mmol/L Na-EDTA, and 0.1% bromophenol blue), boiled for 2 minutes, and loaded onto a 10% SDS polyacrylamide gel. Proteins were electroblotted onto polyvinylidene fluoride membranes (Pall Gelman), blocked with 6% nonfat dry milk in TBST (200 mmol/L Tris-HCl [pH 7.5], 300 mmol/L NaCl, and 0.1% Tween 20) at room temperature for 1 hour, and incubated with 5 μg/mL of the primary human antibodies for Bcl-x_L (rabbit polyclonal, H-62; Santa Cruz Biotechnology) and for Bcl-2 (mouse monoclonal, 124; DAKO) at room temperature for 4 hours. Blots were subsequently washed in TBST and incubated with peroxidase-conjugated antibodies (anti-rabbit or anti-mouse IgG, respectively; Amersham). Bound antibodies were detected with

TABLE 3. Molecular Phenotype Characterization of the Left Ventricular Myocardium of Organ Donors and of Patients With End-Stage HF

mRNA/RNA	Organ Donors	HF Patients		
		No VAD Support	VAD Support	
			Pre-VAD	Post-VAD
Overload-sensitive protein				
Pro-ANP, amol/ng	17±20	105±150*	180±207*	28±28§
Apoptosis-associated proteins				
Bak, amol/mg	301±105	170±93*	158±91*	138±52
Bax, amol/mg	2.1±1.2	2.3±2.3	1.6±0.9	2.1±1.4
Bcl-2, amol/mg	16.4±9.5	18.1±11.9	11.4±9.0	9.3±5.0
Bcl-x _L , amol/μg	215±92	134±57*	144±47†	176±42§
Fas, amol/μg	0.9±0.3	1.2±0.5	1.2±0.3†	1.2±0.5
FasExo6Del, amol/mg	730±420	300±150†	240±120*	250±140
FasExo6Del/Fas, ×10 ⁻³	850±420	250±100*	190±90*	230±90
Mcl-1, amol/ng	3.2±1.5	3.5±1.6	3.5±0.8	4.2±1.1§
Apoptotic DNA fragmentation, RU	0.13±0.11	0.35±0.23*	0.47±0.14*	0.34±0.21
Heart-specific functional proteins				
SR Ca ²⁺ -ATPase, amol/pg	91±41	ND	59±10‡	63±30
Na ⁺ -Ca ²⁺ exchanger, amol/ng	533±257	ND	736±247‡	652±224
SR Ca ²⁺ -ATPase/Na ⁺ -Ca ²⁺ exchanger	198±118	ND	87±33*	103±47
n	10	24	10	

Values are mean±SD. RU indicates relative units (integral area of the 200-bp DNA fragment/μg DNA); ND, not determined.

* $P<0.01$, † $P<0.05$, ‡ $0.1>P>0.05$ vs organ donors. § $P<0.05$, || $0.1>P>0.05$ vs Pre-VAD.

enhanced chemiluminescence detection reagents (Amersham), quantified by use of a laser-densitometer with an imaging system (Molecular Dynamics), and normalized in comparison with the densitometrically determined value of the total protein loading per lane. In control reactions, immunodetection occurred without the primary antibodies. In all cases, control reactions were negative.

Data Analysis

Clinical data and results of molecular investigations are given as mean±SD. Student's paired or unpaired *t* test was used for statistical comparison as appropriate. 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

Data of HF Patients Without VAD Support

Compared with the myocardium of organ donors, the myocardium of HF patients is characterized by an increased mRNA expression of Pro-ANP and a decrease in FasExo6Del, Bcl-x_L, and Bak (Table 3). Furthermore, we observed elevated apoptotic oligonucleosome-sized DNA fragmentation in left ventricles (Table 3, Figure 1A) associated with an increased abundance of apoptotic cardiomyocytes observed by the TUNEL assay (Figure 1B).

Influence of ACE Inhibitor Therapy on Bcl-x_L and Bcl-2 Expression

Whereas patients without therapy by inhibitors of the angiotensin-converting enzyme (ACE) showed low mRNA levels of Bcl-x_L (92.0 amol/μg RNA) and of Bcl-2 (10.1 amol/mg RNA) (n=8), patients under ACE inhibitor treatment (n=16)

revealed partial normalization of Bcl-x_L mRNA (147.8 amol/μg RNA) and Bcl-2 mRNA (20.9 amol/mg RNA) ($P<0.01$ and $P<0.05$, respectively). These mRNA data could be confirmed on the protein level, which was observed by Western blot analysis (Figure 2). In patients treated with ACE inhibitors compared with patients with no ACE inhibitor treatment, the Bcl-x_L protein level was determined to be 1.0 versus 0.51 relative units ($P<0.001$), respectively, and the Bcl-2 protein level was 0.79 versus 0.32 relative units ($P<0.09$), respectively. Furthermore, we observed a significantly positive correlation between Bcl-x_L or Bcl-2 protein and the mRNA levels in the group of all HF patients and donors ($r=0.488$ [$P<0.05$] and $r=0.461$ [$P<0.05$], respectively). However, these HF patients showed no significant left ventricular alterations either in apoptotic DNA fragmentation or in Pro-ANP mRNA expression or ejection fraction depending on the therapy with ACE inhibitors.

Data of HF Patients Supported by VAD

Clinical data of the 10 VAD patients revealed a highly critical hemodynamic state before VAD implantation (Table 1). During the time of ventricular support, a tendency to improved cardiac function could be assumed in these patients from decreasing cardiothoracic x-ray ratios or transthoracic echocardiography. However, these putative improvements could not be exactly quantified because the VAD was not temporarily turned off for measurements of the genuine hemodynamic parameters. This potentially risky procedure was avoided, since this observational study was not designed

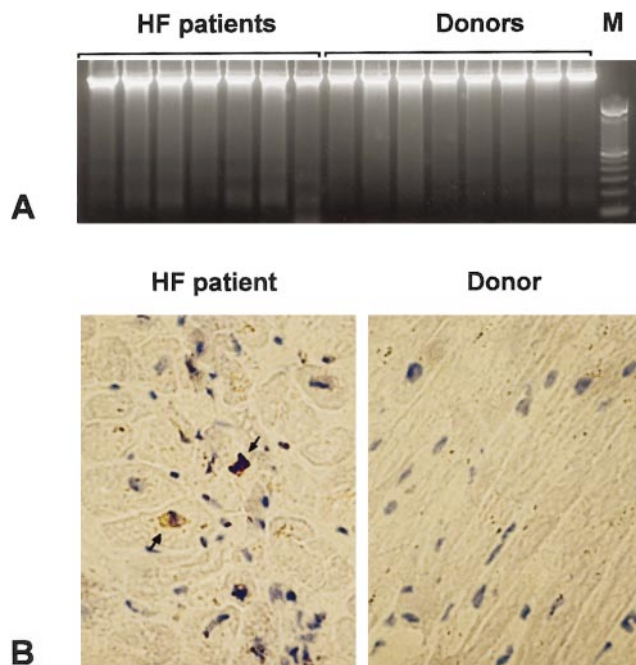


Figure 1. Comparison of left ventricular data from patients with end-stage HF and donors. A, DNA gel electrophoresis of 1.5 μ g DNA indicates apoptotic DNA fragmentation in HF patients. M indicates 100-bp DNA marker. B, Apoptotic cardiomyocyte nuclei detected by TUNEL assay appear brown-stained and are indicated by arrows.

to prove the potential of cardiac recovery under VAD. Nevertheless, shortly before the orthotopic cardiac transplantation, a decrease in pulmonary capillary wedge pressure and an absence of deterioration or even an increase in the cardiac index (determined either by pulmonary artery catheter or transthoracic echocardiography) indicated the beneficial effects on hemodynamic unloading by VAD (Table 1).

Transcription of Pro-ANP

In left ventricular specimens, we observed a decrease in Pro-ANP mRNA expression under hemodynamic unloading approaching the mRNA levels of donor ventricles (Table 3 and Figure 3). This downregulation of Pro-ANP mRNA did not depend on time elapsed on VAD: it reached 23 ± 18 amol/ng RNA in patients with <100 days ($n=4$) and 31 ± 30 amol/ng RNA in patients with >100 days ($n=6$) of VAD support compared with the starting level of 180 ± 207 amol/ng RNA.

Apoptotic DNA Laddering

No or minimal signs of DNA fragmentation could be observed in left ventricles of donors in accordance with

TUNEL-negative cardiomyocytes (Figure 1). The left ventricles of VAD-supported patients showed an intensive DNA fragmentation before VAD unloading. This DNA laddering tended to be attenuated after the time on VAD ($P=0.07$, Tables 1 and 3 and Figure 3).

Transcription of Apoptosis-Associated Genes

Before VAD support of terminally failing hearts, left ventricular transcription of Bcl-x_L, FasExo6Del, and Bak was reduced compared with that in nonfailing left ventricles of organ donors. These data are comparable to those of HF patients without VAD support (Table 3). After VAD unloading, the Bcl-x_L mRNA expression was enhanced by $29 \pm 30\%$ ($P<0.05$), and this improvement revealed a significant dependence on duration of VAD support (Figure 4A). Furthermore, Figure 4A indicates that the left ventricular Bcl-x_L mRNA expression reached the mean value of donor ventricles after ≈ 130 days of VAD unloading.

Similarly, an increase in mRNA expression of FasExo6Del in relation to Fas correlated with the duration of ventricular support in DCM patients (Figure 4B). However, this time-dependent increase in the ratio of FasExo6Del per Fas did not reach the level of increase exhibited by donor ventricles during the period of VAD unloading. In addition, the mRNA level of Mcl-1 was slightly elevated ($20 \pm 26\%$) under VAD support ($P<0.05$). For Bcl-2 and the Bcl-2-related Bak and Bax, we did not observe any transcriptional changes under support by VAD (Table 3). Consequently, the left ventricular mRNA level of Bcl-x_L was increased in relation to its heterodimerization partner Bak by $42 \pm 46\%$ ($P<0.06$).

The left ventricular mRNA expression of the α subunit of the leukocyte-specific adhesion glycoprotein p150.95, an indicator of inflammatory cell infiltration, did not change during time on VAD ($0 \pm 7\%$).

mRNA Expression of Na⁺-Ca²⁺ Exchanger and SR Ca²⁺-ATPase

mRNA expression of the Na⁺-Ca²⁺ exchanger tended to be elevated in the left ventricles of HF patients before VAD support compared with the left ventricles of donor hearts, whereas mRNA of the SR Ca²⁺-ATPase tended to be lowered, and the ratio of SR Ca²⁺-ATPase per Na⁺-Ca²⁺ exchanger was significantly reduced. Under hemodynamic unloading, left ventricular transcription of the SR Ca²⁺-ATPase as well as of the Na⁺-Ca²⁺ exchanger remained at failing levels despite this mechanical support (Table 3).

However, in the left ventricles of 4 patients (Nos. 3, 4, 6, and 8; see Table 1), an improvement of the mRNA ratio of SR Ca²⁺-ATPase per Na⁺-Ca²⁺ exchanger ($\geq 40\%$; ie, larger than

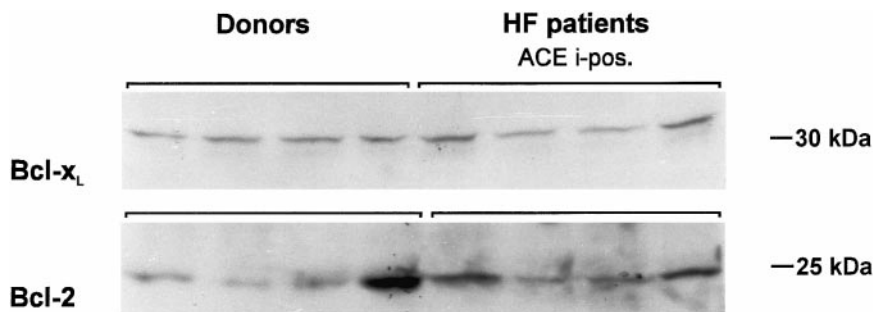


Figure 2. Left ventricular protein expression of Bcl-x_L and Bcl-2 from organ donors and from patients with end-stage HF who were treated with ACE inhibitors (ACE i-pos.). Western blot analysis indicates myocardial Bcl-x_L as 30-kDa and Bcl-2 as 25-kDa protein.

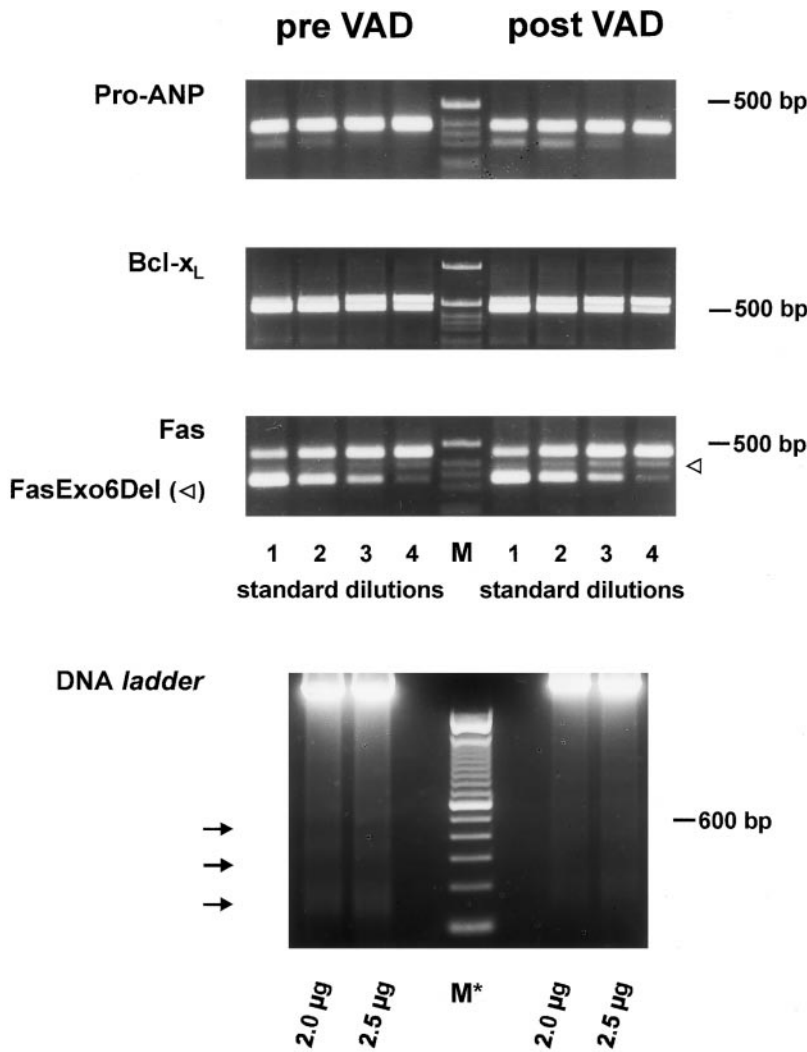


Figure 3. Agarose gel separation of cDNA fragments resulting from standard-calibrated competitive RT-PCR using left ventricular mRNAs for Pro-ANP, Bcl-x_L, Fas, and FasExo6Del before and after VAD support of patient 5 (see Table 1). A weaker intensity of the shorter standard cDNA fragment in relation to target cDNA indicates higher transcription of respective gene. Attenuated left ventricular apoptotic DNA fragmentation under VAD support is shown below. M and M* indicate 100-bp DNA marker.

the SD of pre-VAD values) could be observed. These 4 patients suffered from DCM, and post hoc comparison with the remaining 4 DCM patients without such improvement of the SR Ca²⁺-ATPase/Na⁺-Ca²⁺ exchanger mRNA level under VAD indicated that they also showed more improved gene expressions of antiapoptotic factors. Their myocardial mRNA levels of FasExo6Del were increased by 94±45% (*P*=0.05), of Mcl-1 by 42±9% (*P*=0.07), and of Bcl-x_L/Bak by 59±49% (*P*=0.05) during the time on VAD. Similar elevations were not observed in the other subgroup for FasExo6Del (7±80%), Mcl-1 (9±41%), or Bcl-x_L/Bak (33±52%), although the duration of VAD support was not significantly different in both subgroups (102±52 versus 84±52 days in patients with versus without increased SR Ca²⁺-ATPase/Na⁺-Ca²⁺ exchanger mRNA level). The subgroup without a VAD-induced increase in SR Ca²⁺-ATPase/Na⁺-Ca²⁺ exchanger mRNA expression may have been in a worse condition immediately before VAD implantation: their cumulative dosage of phosphodiesterase inhibitors, needed for positive inotropic treatment, was 564±356 mg milrinone equivalents (in case of the use of enoximone, the enoximone dosage in milligrams was divided by 5.5), and the last monitored pulmonary wedge pressure was determined to be

35±5 mm Hg. These values tended to be lowered in the subgroup with an improved expression of the mentioned genes under VAD: the pulmonary wedge pressure was 25±6 mm Hg (*P*=0.06 versus the other subgroup) and the cumulative milrinone equivalent dosage was 233±191 mg.

Discussion

In the present study, we report that the HF patients who required VAD support exhibited an apoptosis-susceptible myocardial phenotype and an abnormal myocardial mRNA expression of calcium-regulatory proteins before VAD insertion that were comparable to those of other HF patients who were not treated by VAD unloading.

Compared with donor hearts, the hearts of the HF patients exhibited increased cardiac DNA fragmentation with more abundant TUNEL-positive cardiomyocytes; these data are in agreement with the data of Olivetti et al,⁹ which additionally showed that in overloaded human myocardium apoptosis mainly affects cardiomyocytes. Thus, the reduction in myocardial DNA laddering under VAD (Tables 1 and 3) probably reflects the decrease in the ongoing apoptotic loss of cardiac myocytes, because mRNA expression of the leukocyte adhesion molecule p150.95,²³ an indicator of inflammatory cell

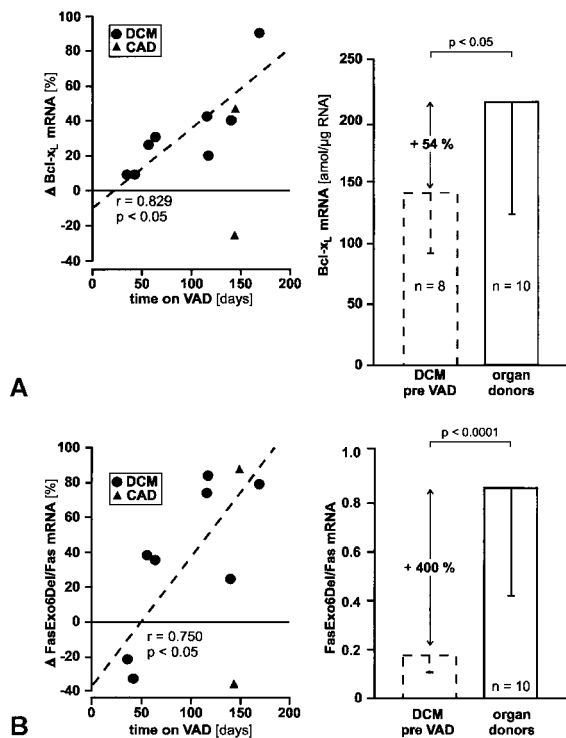


Figure 4. Left ventricular mRNA expression of Bcl-x_L (A) and FasExo6Del/Fas (B) in DCM patients compared with donors (right). The respective left panels of A and B illustrate mRNA increase depending on duration of VAD support. Dotted regression line results from data of DCM patients.

invasion, remained constant during time on VAD. This reduction in myocardial apoptosis occurs in association with upregulation of the apoptosis-preventing determinants Bcl-x_L, Mcl-1, and FasExo6Del/Fas (Table 3 and Figures 3 and 4). The quantitative PCR method revealed that Mcl-1 and Bcl-x_L are highly transcribed Bcl-2 relatives in the human left ventricle. In DCM patients, the time-dependent increase in Bcl-x_L and FasExo6Del/Fas indicates the gradual reinduction of these antiapoptotic regulators under hemodynamic unloading. Since the leukocyte p150.95 mRNA level was not altered under VAD, it is unlikely that a changing myocardial content of inflammatory cells influences the cardiac mRNA expression of apoptosis-associated genes.

The enhanced ventricular Pro-ANP transcription is more rapidly renormalized than is Bcl-x_L or FasExo6Del/Fas, confirming the ventricular level of Pro-ANP mRNA as an indicator of overload.²⁴ Furthermore, this could explain the mRNA upregulation of the antiapoptotic Mcl-1: *in vitro* experiments have shown that ANP treatment of cardiomyocytes increased signs of apoptosis associated with a reduction in Mcl-1 mRNA.²⁵ However, since Mcl-1 mRNA is not altered between donors and excessive Pro-ANP-expressing HF patients, this VAD-associated induction of Mcl-1 mRNA could be a rather transient effect.

The VAD-induced reduction in left ventricular wall tension^{18,19} appears to be more relevant for a decrease in myocardial apoptosis of failing hearts under mechanical support. Distension-mediated myocyte apoptosis has been experimentally associated with a p53-mediated enhancement

of the renin-angiotensin system, a decreased expression of antiapoptotic versus proapoptotic Bcl-2-related proteins,²⁶ and an elevated translation of the Fas receptor.⁷ Furthermore, an increase in cardiomyocyte apoptosis and the changed expression of Bcl-2 relatives by experimental overactivity of ACE could be renormalized by angiotensin 1 receptor blockade²⁷ or by long-term therapy with ACE inhibitors.²⁸ Confirming these experimental data, expressions of Bcl-x_L and Bcl-2 were partially normalized by ACE inhibitor therapy in the failing human myocardium of our patients. Since nearly all VAD patients were treated with ACE inhibitors, VAD unloading has an additionally stimulatory effect on the mRNA expression of the apoptosis-preventing Bcl-x_L, which correlates with the Bcl-x_L protein. Furthermore, after ventricular support of patients who had a coronary heart disease, an upregulation of the Bcl-x_L protein could be observed, too.²⁹ However, the ventricular unloading, either VAD-mediated or ACE inhibitor-mediated, cannot be the sole stimulus: the overload-indicating Pro-ANP mRNA level was identical in the non-VAD-supported group of patients with and without ACE inhibitor therapy.

Although our data indicate partial apoptotic phenotype normalization under VAD support with cardiac apoptosis reduction of the failing myocardium, these results cannot yet provide an assessment of the quantitative relevance of myocyte apoptosis. Furthermore, they cannot indicate the therapeutic potential of assist devices in terminal HF for several reasons: First, the analysis could not be designed as a treatment study. Second, we analyzed myocardium only from those patients who experienced a clinical stabilization under assist device unloading. Not all patients in such a deteriorated state of terminal HF can be successfully stabilized by a VAD.⁵ Third, it must be remembered that the myocardium of some patients may have lost this hypothetical potential for recovery because of irreversible remodeling processes, like fibrosis and scar formation.

Finally, it remains to be stressed that a reduction in apoptosis is only one of several basic mechanisms required for a functional recovery of the failing heart. Therefore, mRNA expressions of SR Ca²⁺-ATPase and Na⁺-Ca²⁺ exchanger were additionally determined. However, the mRNA ratio of SR Ca²⁺-ATPase per Na⁺-Ca²⁺ exchanger was normalized in only a few patients. Since a correlation between impaired mRNA and protein levels of the SR Ca²⁺-ATPase and the decreased SR ⁴⁵Ca²⁺ uptake has been demonstrated in the failing human heart,^{20,30,31} our results are comparable to those of Frazier et al,³² who observed an enhanced myocardial calcium uptake/binding in the SR from some HF patients under chronic VAD support. As mentioned above, the myocardium of only some patients will be capable of renormalizing the expression of calcium-regulatory determinants under VAD for irreversible injuries. Furthermore, VADs are presently used only in patients who cannot be stabilized otherwise, and up to now, only a few selected patients could be weaned from the VAD without a subsequent heart transplantation. The selective use of VADs is necessary because it seems not to be justified to withhold an organ offer from those patients who need it.⁶ It is plausible to postulate that the VAD application in patients with less deteriorated

cardiac function could be more successful. In agreement with this assumption is our observation that the subgroup with VAD-induced improvement of gene expression for antiapoptotic and calcium-handling proteins tends to exhibit a somewhat less deteriorated hemodynamic status immediately before VAD implantation, as can be deduced from a diminished cumulative phosphodiesterase inhibitor dosage and a lower pulmonary wedge pressure.

In conclusion, our findings indicate some potential of VAD as an intervention to interrupt the vicious cycle of distension-induced proapoptotic phenotype shifts, and in the future, VAD may become a treatment option for recovery in a subgroup of patients otherwise undergoing transplantation.

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Myocardial Gene Expression of Regulators of Myocyte Apoptosis and Myocyte Calcium Homeostasis During Hemodynamic Unloading by Ventricular Assist Devices in Patients With End-Stage Heart Failure

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