Progression From Compensated Hypertrophy to Failure in the Pressure-Overloaded Human Heart
Structural Deterioration and Compensatory Mechanisms

Stefan Hein, MD*; Eyal Arnon, MD*; Sawa Kostin, MD; Markus Schönburg, MD; Albrecht Elsässer, MD; Victoria Polyakova, PhD; Erwin P. Bauer, MD; Wolf-Peter Klövekorn, MD; Jutta Schaper, MD

Background—The progression of compensated hypertrophy to heart failure (HF) is still debated. We investigated patients with isolated valvular aortic stenosis and differing degrees of left ventricular (LV) systolic dysfunction to test the hypothesis that structural remodeling, as well as cell death, contributes to the transition to HF.

Methods and Results—Structural alterations were studied in LV myectomies from 3 groups of patients (group 1: ejection fraction [EF] >50%, n=12; group 2: EF 30% to 50%, n=12; group 3: EF <30%, n=10) undergoing aortic valve replacement. Control patients were patients with mitral valve stenosis but normal LV (n=6). Myocyte hypertrophy was accompanied by increased nuclear DNA and Sc-35 (splicing factor) content. ACE and TGF-β were upregulated correlating with fibrosis, which increased 2.3-, 2.2-, and 3.2-fold over control in the 3 groups. Myocyte degeneration increased 10, 22, and 32 times over control. A significant correlation exists between EF and myocyte degeneration or fibrosis. Ubiquitin-related autophagic cell death was 0.5‰ in control and group 1, 1.05 in group 2, and 6.05‰ in group 3. Death by oncosis was 0‰ in control, 3‰ in group 1, and increased to 5‰ (groups 2 and 3). Apoptosis was not detectable in control and group 3, but it was present at 0.02‰ in group 1 and 0.01‰ in group 2. Cardiomyocyte mitosis was never observed.

Conclusions—These structure-function correlations confirm the hypothesis that transition to HF occurs by fibrosis and myocyte degeneration partially compensated by hypertrophy involving DNA synthesis and transcription. Cell loss, mainly by autophagy and oncosis, contributes significantly to the progression of LV systolic dysfunction. (Circulation. 2003;107:984-991.)

Key Words: hypertrophy ■ heart failure ■ structure ■ remodeling ■ hemodynamics

The structural basis of the progression from well-compensated hypertrophy caused by mechanical overload to heart failure (HF) is still largely unknown. It is evident that cardiac remodeling, defined as "genome expression, molecular, cellular, and interstitial changes that are manifested clinically as changes in size, shape and function of the heart after injury" occurs in the chronically pressure-overloaded heart. However, the correlation between morphological alterations and clinical data during the different phases of transition to HF has not yet been described in the human heart.

Krayenbühl and colleagues in 1989 described fibrosis and myocyte enlargement in patients with aortic stenosis (AS) with normal ejection fraction (EF) but elevated left ventricular end-diastolic pressure (LVEDP).They studied cardiac functional recovery after aortic valve replacement (AVR) but not the progression to HF. In end-stage human HF, we reported fibrosis as well as impairment of the myocyte ultrastructure defined as degeneration. Anversa et al (review by Elsässer et al) emphasized cell death, mainly by apoptosis, as one of the key events for the occurrence of failure.

Fibrosis and myocyte damage appear to be the decisive morphological alterations in the remodeling process. The most important regulators of fibrosis are the members of the renin-angiotensin and aldosterone system. In addition, TGF-β is a potent stimulator of fibrosis, as are growth factors and endocrine hormones such as norepinephrine (reviewed by Hein and Schaper). Recent studies in small rodents have shown that the development of fibrosis is prevented in the absence of TGF-β, indicating the crucial fibrosis-promoting role of this cytokine.
Earlier, we described myocyte degeneration and loss in the transition to end-stage HF. In the present study, we investigated patients with AS in different hemodynamic situations ranging from compensatory hypertrophy with intact systolic function to symptomatic HF with depressed systolic function.

We studied the relation between clinical LV parameters and morphological alterations including fibrosis, myocyte degeneration, and cell death. To test the assumption that compensatory mechanisms may be active, we determined the DNA content and the presence of the splicing factor Sc-35.13 The

<table>
<thead>
<tr>
<th>TABLE 1. Clinical Preoperative Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Men/women</td>
</tr>
<tr>
<td>EF, %</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
</tr>
<tr>
<td>PCWP, mm Hg</td>
</tr>
<tr>
<td>PAPmean, mm Hg</td>
</tr>
<tr>
<td>∆Pmean, mm Hg</td>
</tr>
<tr>
<td>Valve orifice, cm²</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
</tr>
<tr>
<td>LV relative WT</td>
</tr>
<tr>
<td>LV mass/m²</td>
</tr>
<tr>
<td>Atrial fibrillation[]</td>
</tr>
<tr>
<td>LV decompensation[]</td>
</tr>
<tr>
<td>Diuretics[</td>
</tr>
<tr>
<td>Digitalis[</td>
</tr>
<tr>
<td>ACE inhibitors[</td>
</tr>
<tr>
<td>β-Blockers[</td>
</tr>
</tbody>
</table>

All patients with regurgitation of aortic/mitral valve ≥1 degree.
Etiology: n=1 bicuspid, n=11 degeneration, n=22 postendocarditis.
*PCWP and PAP pressure in control subjects are elevated because of MS (real control values <10 and <18 mm Hg, respectively).
†P<0.05 compared with control; ‡P<0.05, group 3 vs group 1; all others not significantly different.
§Mitral valve orifice and pressure gradient in control subjects.
||No. of patients.

<table>
<thead>
<tr>
<th>TABLE 2. Morphometric Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Myocyte No./mm²</td>
</tr>
<tr>
<td>Cross-sectional area, μm²</td>
</tr>
<tr>
<td>Myocytes &lt;300 μm², %</td>
</tr>
<tr>
<td>Longitudinal cell area, μm²</td>
</tr>
<tr>
<td>Nuclear area, μm²</td>
</tr>
<tr>
<td>Nuclear/myocyte area</td>
</tr>
<tr>
<td>Ki-67, n/mm²</td>
</tr>
<tr>
<td>CD3, n/mm²</td>
</tr>
<tr>
<td>CD68, n/mm²</td>
</tr>
<tr>
<td>CD45, n/mm²</td>
</tr>
<tr>
<td>Ubiquitin positive, %</td>
</tr>
<tr>
<td>C9 positive, %</td>
</tr>
<tr>
<td>Apoptosis, %</td>
</tr>
<tr>
<td>Myocyte degeneration, %</td>
</tr>
</tbody>
</table>

*P<0.05 vs control, †P<0.05 vs group 1.
final goal of this work was 2-fold: (1) To define the mode of transition of compensated hypertrophy to HF in the pressure-overloaded human heart with emphasis on the role of cell death: autophagy associated with the ubiquitin-proteasomal pathway, oncosis (necrosis is cellular breakdown after cell death has occurred), and apoptosis, and (2) to determine the correlation between preoperative/postoperative clinical data and morphological findings, which might determine the potential for complete postoperative recovery.

Methods

Patients
Thirty-four patients with isolated AS underwent clinical evaluation (Table 1) and were subdivided into 3 different groups on the basis of EF determined by quantitative echocardiography at the time of admission: group 1, EF >50% (n=12); group 2, EF 50% to 30% (n=12); group 3, EF <30% (n=10). All patients underwent surgical AVR and postoperative examination (identical n per group). Six patients with mitral stenosis (MS) with normal EF served as control subjects. The institutional ethics committee approved the study, and all patients gave informed consent.

Tissue Sampling
During open heart surgery, myectomy samples weighing ~30 to 80 mg were removed from the LV septum, immediately frozen in liquid nitrogen, and stored at −80°C. In MS, samples from papillary muscles were obtained. In addition, small samples were fixed in buffered glutaraldehyde for electron microscopy.

Electron Microscopy
The samples were embedded in Epon, following a standard protocol. Ultrathin sections were double-stained with uranyl acetate and lead citrate before examination in a Philips CM 10. Myocyte degeneration, defined as loss of contractile elements and disorganization of ultrastructural organelles, was evaluated quantitatively by two observers blinded to the patient group.

Immunolabeling and Confocal Microscopy
Cryosections 5 μm thick were air-dried and fixed either with paraformaldehyde or acetone. Primary antibodies were fibronectin (rabbit polyclonal, ICN), TGF-β1, ACE (Chemicon), CD3 (lymphocytes), CD31 (endothelial marker, PECAM), CD45 (panleukocytes), CD68 (macrophages), and Ki-67 (DNA synthesis) (all Dako), vinculin, sarcomeric α-actinin, and Se-35 splicing factor (all Sigma), ubiquitin for autophagic cell death, (Zymed Laboratories), complement 9 (C9, Serotec) for oncosis, and the TUNEL method for apoptosis (Roche). The specificity of all antibodies was verified by omission of primary antibodies.

The secondary detection system was biotinylated anti-mouse or anti-rabbit IgG (Biotrend) either directly conjugated with Cy-2 or Cy-3 or unconjugated followed by fluorescein-isothiocyanate (FITC)-linked streptavidin (Amersham). Myocyte identification was done with TRITC-labeled phalloidin (Sigma). Nuclei were stained with TOTO-3 (Molecular Probes). Picture acquisition was performed with a Leica confocal microscope (CLSM); data were transferred to a Silicon Graphics workstation for further processing and recording (Bitplane software).

Morphometry
Myocyte degeneration was evaluated in the confocal microscope from α-actinin–stained sections at a magnification of ×400. The total myocyte number was determined and degeneration calculated as percentage. Myocyte cross-sectional and longitudinal cell areas were determined directly in the confocal microscope from at least 120 myocytes per section by delineating vinculin-stained myocytes.

Fibrosis and Numerical Densities
Fibrosis was quantified from fibronectin-stained sections from 5 different fields of vision randomly chosen and expressed as percentage of total myocardium. Capillary density was determined from 5 different fields of PECAM-stained sections and calculated (as n/mm²). The same was done for ACE-positive microvessels as well as for CD3, CD45, CD68, and Ki-67. All densities were determined at a microscopic magnification of ×250.

Cell Death
Numbers of ubiquitin-, C9-, and TUNEL-positive cells were obtained from the entire section. Section size and number of myocytes per mm² were determined, and percentages of positive myocytes were calculated. The total number of myocytes evaluated per patient varied between 2748 and 5976.

Fluorescence Intensity by Confocal Microscopy
The immunolabeling procedures for TGF-β1 were carried out under identical conditions, including the microscopic magnification of ×400. Confocal settings were kept constant, and quantification of TGF-β1 was performed by measurements of fluorescence intensity.
by using a range of 0 to 255 values. Arbitrary units were calculated per unit surface area (AU/mm²).

**FEULGEN Staining and Quantitative Evaluation**

Nuclear DNA was stained by the FEULGEN method. All sections were stained simultaneously under exactly the same protocol. Quantitative evaluation of the nuclear DNA content (total amount times nuclear area) and concentration (DNA/nuclear area) was done with the CLSM at a magnification of 400. At least 400 nuclei per sample were measured. The same procedure was followed for Sc-35. Nuclear area was automatically obtained from measurements of FEULGEN staining.

**Statistics**

All data are presented as mean±SEM. Differences by unpaired t test, ANOVA, Bonferroni, or Kruskal-Wallis were considered significant when \( P<0.05 \).

**Results**

**Myocyte Hypertrophy and Degeneration**

Myocytes showed hypertrophy. The cross-sectional and longitudinal cell areas were increased in all groups compared with the control (MS) group (Table 2). Interestingly, the number of atrophied myocytes, defined as cells with a cross-sectional area <300 μm², was rather large (Table 2).

Subcellular changes in myocytes by electron and confocal microscopy were reduction of sarcomeres, occurrence of myelin figures and autophagic vacuoles, numerous poly- somes, and nuclei of bizarre shape. The percentage of myocyte degeneration/total number of myocytes was slightly increased in group 1 and significantly elevated in groups 2 and 3 (Figure 1 and Table 2).

Ubiquitin-related autophagic cell death and oncosis increased with depressed EF (Figure 1 and Table 2). In group 3, with severe LV dysfunction, the prevalence of myocytes undergoing autophagic and oncosic death was ≈5% and 4%, respectively, whereas classic apoptosis was detected in <1 per 10,000 myocytes.

**Fibrosis**

Fibrosis was already significantly increased in group 1 as compared with control; it remained unaltered in group 2 and was elevated to almost 40% in group 3 (Figure 2, A through C). A significant correlation exists between the degree of fibrosis and myocyte degeneration (Figure 2D).

**Hemodynamic Status**

Significant pressure overload with increased LVSP was present in all patients as the result of severe AS, with orifice surface ranging between 0.96 to 0.22 cm². The earliest changes caused by chronic pressure overload were an elevation of LVEDP and wall thickness. With decreasing EF, a further increase of LVEDP and as sign of pulmonary congestion an elevation of pulmonary artery pressure was found. Maximal levels of LVSP, ΔP, and relative wall thickness were found in group 1 and decreased in group 3. LV mass tended to be higher in the patients with AS compared with control (MS) patients. Atrial fibrillation occurred more frequently in group 3 than in the other groups.

There was a good correlation between percentage of degenerated myocytes and EF as well as correlations between percentage fibrosis and both EF and LVEDP (Figure 3, A through C). Postoperative evaluation at 2.6±1.4 years after AVR showed that EF was within normal range in group 1 and 2 patients; in contrast, group 3 patients showed continued LV systolic dysfunction associated with persistent depression of

![Figure 2. Fibronectin staining (red, nuclei blue). A, Normal myocardium shows fine septa between unstained myocytes; B, severe fibrosis with few myocytes; C, fibrosis is already severe in group 1; D, significant correlation exists between fibrosis and myocyte degeneration.](http://circ.ahajournals.org/DownloadedFrom)
New York Heart Association functional class (Figure 3, D and E).

**Inflammation Markers**
There was a 3-fold increase in leukocytes (CD45) but only a slight increase in the number of lymphocytes (CD3) and macrophages (CD68) situated in the perivascular and interstitial space (Table 2).

**ACE, TGF-β, and Capillary Density**
The number of CD31-positive microvessels was reduced (Figure 4C). ACE was present in the endothelium of microvessels (Figure 4A) and increased in all groups when expressed as a percentage of capillary density (Figure 4C). TGF-β, localized in fibroblasts and macrophages (Figure 4B), was elevated in all groups (Figure 4D).

**Nuclear DNA and Sc-35**
The content (AU times nuclear area) of DNA and SC-35 was increased in all groups, but the concentration (AU/nuclear area) remained unchanged (Figure 5, A through D). However, since the ratio of nucleus/cell area was decreased (Table 2), the elevation of content is insufficient for the enlarged myocytes. The number of Ki-67–positive myocytes and of binucleated myocytes was unchanged in all groups (Table 2).

**Discussion**
In this study, we present evidence that a close correlation exists between cardiac function and myocardial morphology in patients with AS. With worsening of fibrosis and myocyte degeneration, LVEDP increases and later EF decreases. This suggests that a structure-function relation leading to HF is present in human pressure overload. This correlation is also
reflected in the degree of postoperative recovery, which was incomplete in patients group 3. This should be taken into account for the decision of surgical intervention.

Fibrosis is an early morphological alteration in patients with AS. It is a major determinant of diastolic dysfunction and systolic pumping capacity,\textsuperscript{17} and it is one of the structural substrates for arrhythmogenicity, thus playing a major role for sudden death and the progression of HF.\textsuperscript{18} This is in agreement with Krayenbühl et al.,\textsuperscript{2} who performed serial observations in the same patients and found a partial regression of severe fibrosis 6 to 7 years after AVR, which is unlikely to occur in the patients of group 3 of this study.

Figure 4. ACE and TGF-\(\beta_1\) (green, myocytes red, nuclei blue). A, ACE is localized in numerous microvessels. B, TGF-\(\beta_1\) is present in fibroblasts and extracellular matrix. C, Number of ACE-positive microvessels is significantly elevated in group 1 and remains almost constant when expressed as percentage of total number of capillaries. D, TGF-\(\beta_1\) increases in later stages of hypertrophy.

Figure 5. DNA and Sc-35 labeling (myocytes red, nuclei green for either DNA [A] or Sc-35 [B]). Yellow dots are lipofuscin granules. A, Typical staining pattern for DNA in nuclei. B, Sc-35 localization. C, Nuclear content of Sc-35 and DNA. D, Nuclear concentration of Sc-35 and DNA.
Inflammation was of low grade and reflected in the increase in leukocytes and macrophages observed. These may be responsible for the increase in cytokine production (TGF-β1, TNF-α, interleukin family) that accounts not only for the inflammatory response but also for the formation of reactive fibrosis.

Previous studies of myocardium from patients with AS have shown that ACE and TGF-β1 were upregulated on the mRNA and protein level.19 Because TGF-β1 was upregulated in group 1, we suggest that TGF-β1 is one of the major determinants of fibrosis progression, thus confirming studies by others (reviewed in Reference 20).20 ACE was elevated in group 1. De novo synthesized angiotensin II locally released by the action of ACE regulates TGF-β1 production, and it is this fibrogenic cytokine that regulates the collagen turnover of fibroblasts.9 The present findings corroborate the hypothesis that angiotensin release by the action of ACE is stimulated by a paracrine mechanism involving TGF-β1 as a mediator.21

Myocyte Degeneration
The degree of degenerative injury of cardiomyocytes increased with the development of HF. The term degeneration was chosen to emphasize involvement of all cellular organelles in a chronic and most probably slow process of degradation that finally results in cellular atrophy, myocyte death, and replacement fibrosis. The severity of changes exceeded those typically observed in myocardium from patients with dilated cardiomyopathy. The significant correlation between myocyte degeneration with fibrosis and with EF suggests a mutual influence of cardiac structure and function.

DNA Replication, Transcription, and Lack of Mitosis
Compensatory mechanisms were, however, observed as well. The increase in SC-35, an RNA splicing factor, indicates ongoing transcription in all groups. SC-35 belongs to the group of non-snRNP factors and is required for the first step of splicing and spliceosome assembly.22 We would like to postulate that the presence of SC-35 indicates that the cardiomyocytes, even when damaged, are viable and capable of transcription and translation.

This assumption is reinforced by the DNA data presented in this report, which confirm earlier experimental studies.23,24 The ability of the human myocyte to increase its DNA content avoids “dilution” of the DNA in the enlarged cells and permits DNA repair, thus allowing for a significant hypertrophic response. However, since the nucleus/cell volume ratio is disturbed, the amount of DNA is not sufficient to sustain the transcriptional levels required by the enlarged cell volume and cellular exhaustion will be the consequence. Data published by other groups support the notion that DNA synthesis takes place in the adult mammalian heart,24 and this is strongly suggested by our own data as well.

The cell-cycle-associated nuclear nonhistone Ki-67 protein is expressed in all active phases of the cell cycle but not in quiescent G0 cells.25 It is more abundant in DNA synthesis and mitosis but it might also be observed when DNA synthesis is inhibited.26 The number of binucleated myocytes did not increase with hypertrophy, and therefore we assume that the presence of Ki-67-positive myocytes reflects DNA replication for maintenance of the DNA content/myocyte volume ratio and for DNA repair but not mitotic nuclear division,26 confirming results by other groups.27,28

Mitotic figures indicative of a putative regeneration process were never observed. This contrasts with another study29 but confirms earlier work in human and animal tissue.30 Since cardiomyocyte mitosis was completely absent, the present data implicate that myocyte degeneration will lead to final cell loss.

Ubiquitin-related autophagic cell death13,14 and oncosis31 appear to be more important than apoptosis, which occurred at an extremely low rate. Our data suggest that ubiquitin binds contractile or membrane proteins destined for degradation but that because of proteasomal insufficiency, the complexes are accumulated and might cause nuclear fragmentation (unpublished). Myocytes therefore exhibit large areas with loss of cross-striated sarcomeres. Cells will disintegrate and will be replaced by fibrosis. Narula’s concept16 that activated caspase causes myocyte protein degradation without nuclear DNA fragmentation might be important in that regard and will be pursued in further studies. Single-cell oncosis appears to originate from reduced coronary flow reserve and diffusion disturbances caused by fibrosis and the reduction of capillary density.

On the basis of the data presented here, we depict in Figure 6 a model of the major adaptation to pressure overload. It may be concluded that reduction of cardiac function occurs when mechanisms such as DNA repair and synthesis as well as SC-35 expression are partially or totally exhausted, when myocyte death occurs and fibrosis will have reached a certain degree. These changes will affect an increasing number of myocytes and HF will finally occur. A self-perpetuating process of myocyte degeneration, cell death, and replacement fibrosis will be maintained, even when excessive afterload will return to normal after AVR. This chronic cycle will lead to further impairment of LV function and poor prognosis.
References


Progression From Compensated Hypertrophy to Failure in the Pressure-Overloaded Human Heart: Structural Deterioration and Compensatory Mechanisms

Stefan Hein, Eyal Arnon, Sawa Kostin, Markus Schönburg, Albrecht Elsässer, Victoria Polyakova, Erwin P. Bauer, Wolf-Peter Klövekorn and Jutta Schaper

_Circulation_. 2003;107:984-991; originally published online February 10, 2003;
doi: 10.1161/01.CIR.000051865.66123.B7

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 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/107/7/984

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/