Roles of cyclooxygenase-2 and phosphorylated Akt^(Thr308) in cardiac hypertrophy regression mediated by left-ventricular unloading

Jeremias Wohlschlaeger, MD,^{a,*} Klaus Jürgen Schmitz, MD,^{a,*} Jenci Palatty,^a Atsushi Takeda, MD,^b Nobuakira Takeda, MD,^c Christian Vahlhaus, MD,^e Bodo Levkau, MD,^d Jörg Stypmann, MD,^e Christof Schmid, MD,^f Kurt Werner Schmid, MD,^a and Hideo Andreas Baba, MD^a

Objectives: Cyclooxygenase-2 is associated with cardiac hypertrophy during chronic heart failure and is regulated through the PI3K/Akt pathway. Cyclooxygenase-2-induced cell growth through Akt phosphorylation was demonstrated in vitro. In chronic heart failure, left ventricular assist devices lead to hypertrophy regression and molecular changes. Therefore, the expression of cyclooxygenase-2, phosphorylated Akt (p-Akt), and p-Erk 1/2, as well as cardiac hypertrophy before and after left ventricular assist device insertion, was investigated.

Methods: In myocardial tissue before and after left ventricular assist device insertion, the expression of cyclooxygenase-2, p-Akt^(Thr308), p-Akt^(Ser473), and p-Erk 1/2 was demonstrated by immunohistochemistry and quantified by morphometry. Colocalization of cyclooxygenase-2 and p-Akt^(Thr308) was investigated by immunodoublestaining.

Results: A significant decrease of cyclooxygenase-2, p-Akt^(Thr308), p-Akt^(Ser473), and p-Erk 1/2 protein expression and hypertrophy regression was observed after left ventricular assist device insertion. A significant correlation between cyclooxygenase-2 and p-Akt^(Thr308) expression, as well as between cyclooxygenase-2 expression and cardiomyocyte diameter, was observed before, but not after, left ventricular assist device insertion. Only cyclooxygenase-2-positive cardiomyocytes showed significant hypertrophy regression on unloading. Sarcoplasmic colocalization of cyclooxygenase-2 and p-Akt^(Thr308) is present before left ventricular assist device insertion and is decreased after unloading, whereas the normal myocardium is completely devoid of it.

Conclusions: Left ventricular assist device treatment is associated with a significant decrease of cyclooxygenase-2, p-Akt^(Thr308), p-Akt^(Ser473), and p-Erk 1/2, and cardiac hypertrophy regression of cyclooxygenase-2-positive cardiomyocytes. The significant correlation and colocalization in cardiomyocytes of cyclooxygenase-2 and p-Akt^(Thr308) before left ventricular assist device insertion suggests a cross-talk between the 2 molecules in the progression of cardiac hypertrophy, which is reversibly regulated by the left ventricular assist device.

From the Department of Pathology and Neuropathology, University Hospital Essen, University of Duisburg-Essen,^a Germany; Faculty of Health Science, School of Physical Therapy, Gumma Paz College,^b Gumma, Japan; Department of Internal Medicine, Jikei University,^c Tokyo, Japan; Division of Pathophysiology, Department of Internal Medicine, University Hospital Essen, University of Duisburg-Essen,^d Germany; Department of Cardiology and Angiology, University Hospital Münster,^e Germany; and Department of Thoracic and Cardiovascular Surgery, University Hospital Münster,^f Germany.

This study was supported by the Deutsche Forschungsgemeinschaft to H. A. Baba (Ba1730/9-1), C. Schmid, and C. Vahlhaus (Va156/5-2).

Received for publication May 10, 2006; revisions received June 29, 2006; accepted for publication July 31, 2006.

Reprint requests: Hideo Andreas Baba, MD, Institute of Pathology, University Hospital of Essen, University of Duisburg-Essen, Hufelandstr. 55, 45122 Essen, Germany (E-mail: hideo.baba@medizin.uni-essen.de).

*J. W. and K. J. S. contributed equally to the study.

J Thorac Cardiovasc Surg 2007;133:37-43
0022-5223/\$32.00

Copyright © 2007 by The American Association for Thoracic Surgery

doi:10.1016/j.jtcvs.2006.07.042

When various stimuli impose increased biomechanical stress, the myocardium reacts by enlarging individual cardiomyocytes, resulting in hypertrophy. Although salutary at the beginning by normalizing wall tension, this condition ultimately instigates an unfavorable outcome with progression to chronic heart failure (CHF) or sudden cardiac death. In response to diverse load conditions, cardiomyocytes undergo hypertrophy or apoptosis ("cardiac remodeling").¹ The only curative treatment for terminal CHF is cardiac transplantation. Left ventricular assist devices (LVADs) are used to support patients until transplantation and/or to

Abbreviations and Acronyms

CHF = chronic heart failure
 COX = cyclooxygenase
 LVAD = left ventricular assist device

restore basic cardiac function.² LVADs lead to decreased cardiac size and dilation,³ reflected by decreased cardiomyocyte diameters,^{4,6} length, and volume.⁶ Moreover, there are changes of molecular systems involved in cardiomyocyte growth and apoptosis ("reverse remodeling").⁷

CHF is associated with the induction of inflammatory factors including prostanoids, which exert diverse functional and morphologic effects on cardiomyocytes. Prostanoids (prostaglandins and thromboxane A₂) are the metabolic products of arachidonic acid via the cyclooxygenase (COX) pathway. Today, 3 forms of COX are recognized:⁸ (1) constitutive COX-1; (2) COX-3, an alternatively spliced form of COX-1; and (3) COX-2, which is rapidly induced in response to various stimuli including cytokines⁹ such as tumor necrosis factor- α and hypoxia,¹⁰ both considered hallmarks of CHF.¹¹ Consequentially, increased expression of COX-2 was demonstrated in the failing myocardium.¹² COX-2 expression is regulated through multiple signaling pathways,¹³ including the PI3K/Akt pathway, which has emerged as a major player in cell growth and apoptosis.¹⁴ Overexpression of COX-2-induced Akt phosphorylation at Thr308, but not at Ser473, was demonstrated in vitro, indicating that the COX-2-induced growth stimulus is mediated mainly by phosphoinositide-dependent protein kinase-1-controlled Akt phosphorylation.¹⁵ Akt itself was suggested as a promoter of cardiac hypertrophy.¹⁶

The aim of this study was to investigate whether COX-2 is reversibly regulated in CHF by LVADs. Moreover, the roles of phosphorylated Akt (p-Akt) and Erk 1/2 in the regulation of COX-2 were analyzed in both CHF and "reverse remodeling." Because both COX-2 and Akt are involved in cardiomyocyte growth, the correlation between these proteins and cardiac hypertrophy was determined, and a potential colocalization was investigated by immuno-doublestaining.

Materials and Methods**Patients**

A total of 35 patients (25 patients with Novacor N100; Baxter Healthcare Corporation, Novacor Division, Oakland, Calif; 3 patients with Heartmate 1000 IP, Thermo Cardiosystems Inc, Woburn, Mass; and 7 patients with MicroMed DeBakey axial flow pump system, MicroMed Technologies Inc, Woodlands, Tex) who underwent LVAD implantation for end-stage CHF as a bridge to transplantation were investigated. Dilated cardiomyopathy was diagnosed in 20 patients, 13 with ischemic cardiomyopathy, 1 with myocarditis, and 1 with congenital heart disease (tetralogy of Fallot). The patients' mean age was 43.55 years (median: 46 years;

range: 18-58 years). The mean duration of LVAD implantation was 167.86 days (median: 164 days; range: 14-298 days). Six healthy donor hearts that were not used for transplantation served as controls.

Determination of Cardiomyocyte Diameter

Slides of each individual tissue removed at the time of LVAD implantation (before LVAD from the cardiac apex) and at the time of transplantation (after LVAD from the ventricular wall cranial to the apex to avoid sampling of scar tissue) were stained with periodic acid-Schiff reaction. With an image analysis program (KS 300, Zeiss, Oberkochen, Germany), the diameters of at least 100 cardiomyocytes were determined in randomly selected visual fields at a 400-fold magnification.

Moreover, the diameters of COX-2-positive and negative cardiomyocytes were selectively determined before and after LVAD implantation in randomly chosen myocardial regions.

Immunohistochemistry and Morphometric Evaluation of COX-2, p-Akt, and p-Erk 1/2

4- μ m-thick sections of formalin-fixed and paraffin-embedded tissues were dewaxed and rehydrated according to standard procedures. The specimens were heated in citric acid buffer at 97°C for antigen retrieval and incubated with monoclonal antibodies directed against COX-2 (monoclonal antibody, DCS, Hamburg, Germany), p-Akt^{Thr308}, p-Akt^{Ser473} (polyclonal antibodies, Santa Cruz Biotechnology, Santa Cruz, Calif), and p-Erk 1/2 (polyclonal antibody, Cell Signalling, Cummings). For COX-2, cardiomyocytes were considered positive when a moderate to strong cytoplasmic staining was observed. For p-Akt^(Thr308 and Ser473), cardiomyocytes were considered positive when there was a moderate to strong nuclear staining or both nuclear and cytoplasmic staining. Cardiomyocytes with a moderate to strong nuclear staining for p-Erk 1/2 were regarded as positive.

With an image analysis program, the numeric density of positive cells per visual field with a defined size was determined following the rules of the forbidden and permitted lines. Counts were selectively performed in subepicardial, midmyocardial, and subendocardial areas in 7 randomly selected visual fields in each area and patient.

Immunofluorescence Doublestaining of COX-2 and p-Akt^(Thr308)

Dewaxed slides were treated for antigen retrieval as described above, incubated with antibodies against COX-2 and p-Akt^(Thr308), and visualized by secondary antibodies coupled to Cy3 and fluorescein isothiocyanate, respectively. Nuclei were highlighted by DAPI. Lipofuscin pigments were removed by application of a Sudan black B stain to avoid autofluorescence. Colocalization of COX-2 and p-Akt^(Thr308) was investigated by laser scan microscopy (Zeiss LSM 510, Carl Zeiss Inc, Thornwood, NY) using appropriate filters.

Statistical Analysis

All data were analyzed and expressed as mean $\pm$ standard error of the mean and depicted as box plots. For evaluation of statistical significance, the nonparametric Wilcoxon test for paired samples

Figure 1. Significant decrease of cardiomyocyte diameter by LVAD as calculated by nonparametric Wilcoxon test (*left*). Significant difference between post-LVAD specimens compared with controls as calculated by post hoc analysis according to Duncan (*right*). Data (box plots). Outliers (circles). Significant differences (horizontal bars). Note that cardiomyocyte diameters after LVAD insertion remain higher than in control hearts (A). Significant decrease of COX-2–positive cardiomyocytes after LVAD insertion (*left*). No significant difference between COX-2–negative cardiomyocytes before and after LVAD insertion (*right*) (Wilcoxon test) (B). Significant decrease of cardiomyocytes with expression of COX-2 (C) and p-Akt^(Thr308) (D) after LVAD insertion compared with controls. Data (box plots). Outliers (circles). Significant differences (horizontal bars).

and bivariate correlation analysis according to Spearman were used (Statistical Package for the Social Sciences, SPSS Inc, Chicago, Ill). Intergroup differences between the groups and controls were calculated by one-way analysis of variance followed by post hoc analysis according to Duncan.

Results

Cardiomyocyte Diameter

A significant decrease of general cardiomyocyte diameter was observed after mechanical support ($P = .009$) as calculated by the nonparametric Wilcoxon test (Figure 1, A). The mean cardiomyocyte diameter was $21.25 \mu\text{m}$ (median: $20.84 \mu\text{m}$; range: $4.88\text{--}33.01 \mu\text{m}$) before LVAD insertion and decreased to a mean diameter of $19.39 \mu\text{m}$ (median: $18.86 \mu\text{m}$; range: $9.95\text{--}30.46 \mu\text{m}$) with unloading. The mean diameter of the controls was $15.56 \mu\text{m}$ (median: $16.72 \mu\text{m}$; range: $10.99\text{--}18.05 \mu\text{m}$). However, when post hoc testing according to Duncan was applied, the differences in diameters between tissues obtained before and after LVAD implantation were not significant. When specifically comparing the diameters of COX-2–positive cardiomyocytes before

and after unloading, a significant decrease was observed ($P = .016$), whereas the COX-2–negative cardiomyocyte diameters were not significantly decreased (Figure 1, B). The mean diameter of COX-2–positive cardiomyocytes before LVAD insertion was $23.06 \mu\text{m}$ (median: $22.18 \mu\text{m}$; range: $15.57\text{--}33.01 \mu\text{m}$) and decreased to $20.14 \mu\text{m}$ after LVAD insertion (median: $19.27 \mu\text{m}$; range: $11.65\text{--}30.01 \mu\text{m}$), whereas the mean diameter of COX-2–negative cardiomyocytes before LVAD insertion was $19.77 \mu\text{m}$ (median: $19.07 \mu\text{m}$; range: $12.6\text{--}30.42 \mu\text{m}$) and regressed to $18.75 \mu\text{m}$ after unloading (median: $18.48 \mu\text{m}$; range: $9.95\text{--}30.46 \mu\text{m}$).

Numeric Density of COX-2–Positive Cardiomyocytes

A significant decrease of COX-2–positive cardiomyocytes ($P < .001$) after LVAD insertion was observed. The mean number of COX-2–positive cardiomyocytes was 5.59 per visual field (median: 5.45; range: 0.45–12.77) before LVAD insertion and decreased to 2.28 (median: 1.8; range: 0.15–8.7) after unloading (Figure 1, C). COX-2 expression after LVAD support reached levels similar to the control hearts

Figure 2. Significant decrease of cardiomyocytes with expression of p-Akt^(Ser473) (A) and p-Erk 1/2 (B) after LVAD insertion. Data (box plots). Outliers (circles). Significant differences (horizontal bars). Note that p-Erk 1/2, although significantly reduced, does not decrease to the level of controls (post hoc analysis according to Duncan). Significant positive correlation between COX-2 and p-Akt^(Thr308) protein expression (C) and COX-2 expression and cardiomyocyte diameter before LVAD insertion (D).

as calculated by post hoc analysis (mean count: 0.85; median: 0.74; range: 0.0-1.77).

Numeric Density of p-Akt^(Thr308)-Positive Cardiomyocytes

The number of cardiomyocytes with positive signals for p-Akt^(Thr308) was significantly decreased after LVAD insertion ($P < .001$). The mean count of positive cardiomyocytes was 3.76 per visual field (median: 3.6; range: 1.1-8.3) before LVAD insertion and decreased to 2.31 (median: 2.37; range: 0.10-4.25) after unloading. The mean count in the controls was 2.87 (median: 2.47; range: 1.57-5.93) (Figure 1, D). Post hoc analysis showed a decrease of p-Akt^(Thr308) to the level of controls.

Numeric Density of p-Akt^(Ser473)-Positive Cardiomyocytes

The number of cardiomyocytes staining positively for p-Akt^(Ser473) was significantly decreased after LVAD insertion ($P < .001$). The mean number of positive cardiomyocytes was 3.98 per visual field (median: 3.97; range: 1.20-6.7) before LVAD insertion and decreased to 2.61 (median: 2.45; range: 0.0-5.9) after LVAD insertion.

The mean count in the controls was 2.27 (median: 2.87; range: 0.33-3.77). Post hoc analysis demonstrated a decrease of p-Akt^(Ser473)-positive cardiomyocytes to the level of controls (Figure 2, A).

Numeric Density of p-Erk 1/2-Positive Cardiac Myocytes

Before LVAD insertion, the mean density of cardiomyocytes with positive nuclear staining for p-Erk 1/2 was 2.38 per visual field (median: 2.4; range: 0.0-4.20) and decreased to 1.03 (median: 1.05; range: 0.00-2.75) after mechanical support ($P < .001$) (Figure 2, B). The mean count in the controls was 0.13 (median: 0.03; range: 0.0-0.53); therefore, albeit significantly reduced, p-Erk 1/2 levels did not reach the level of the controls after mechanical support as calculated by post hoc analysis.

By analyzing different regions of the myocardium (subepicardial, midmyocardial, and subendocardial), the immunoreexpression of any of the parameters investigated did not show a zonation (data not shown). In addition to cardiomyocytes, immunoreactivity for COX-2, p-Akt^(Thr308), p-Akt^(Ser473), and Erk 1/2 was observed in endothelial and inflammatory cells and fibroblasts.

Correlation Between COX-2 and p-Akt^(Thr308) Protein Expression

A significant positive correlation between the protein expression of COX-2 and p-Akt^(Thr308) was noted in tissue before LVAD implantation, that is, in CHF ($r = 0.485$; $P < .01$) (Figure 2, C). On investigation of selective, different myocardial areas, this correlation was observed only in the subendocardial area ($r = 0.545$; $P < .01$). However, no correlation between the 2 proteins was observed in tissues obtained after LVAD insertion. Moreover, there was no correlation between COX-2 and p-Akt^(Ser473) or p-Erk 1/2 expression before and after LVAD insertion.

Correlation Between COX-2 Expression and Cardiomyocyte Diameter

Before LVAD implantation, a significant positive correlation between the protein expression of COX-2 and cardiomyocyte diameter was demonstrated ($r = 0.420$; $P < .05$) (Figure 2, D). No such correlation was observed after LVAD implantation. Moreover, a significant correlation between the decrease of COX-2–positive cardiomyocytes and the percentage of reduction of COX-2–positive cardiomyocyte diameters was noted ($r = 0.421$; $P < .05$). No such correlation was found with COX-2–negative cardiomyocytes. Furthermore, there was no correlation between the expression of p-Akt^(Thr308) or p-Erk 1/2 either before or after LVAD implantation.

There was no correlation between the decrease of protein expression of COX-2 or p-Akt^(Thr308) or between the decrease in cardiomyocyte diameter and the underlying cause of CHF. Moreover, no differences in the decrease of the parameters examined could be demonstrated depending on the type of LVAD implanted. Furthermore, no differences with regard to the duration of the LVAD support and decrease of COX-2, p-Akt^(Thr308), and cardiomyocyte diameters could be outlined (data not shown).

Colocalization of COX-2 and p-Akt^(Thr308) in Cardiomyocytes

In a subset of cardiomyocytes, COX-2 and p-Akt^(Thr308) could be detected in the same cell. In some cardiomyocytes an overlay of fluorescein isothiocyanate and Cy3 signals resulting in yellow signals was observed (online Figure 3, A–D). The majority of cardiomyocytes investigated expressed p-Akt^(Thr308) or COX-2. After LVAD insertion, colocalization of the proteins could scarcely be observed. The controls did not show colocalization.

Discussion

A consistent finding after LVAD insertion is a decrease in cardiomyocyte diameters, length, and volume.^{4–6} A chronic inflammatory process with induction of COX-2 contributing to the progression of CHF could be outlined,^{10,11} suggesting that COX-2 is a key element in the inflammatory component

Figure 3. Immunofluorescence doublestaining. Failing myocardium before LVAD insertion: nuclei highlighted in blue (DAPI) (A). p-Akt^(Thr308) highlighted in green (fluorescein isothiocyanate) predominantly in the cardiomyocyte nuclei (green arrows) (B). COX-2 highlighted in red by Cy-3 with granular cytoplasmic distribution (red arrows) (C). Merged images demonstrating colocalization and cytoplasmic overlay of COX-2 and p-Akt^(Thr308) in a subset of cardiomyocytes (yellow arrows) (D).

of CHF.¹² Both COX-2 and Akt have been associated with the progression of cardiac hypertrophy. In several animal models with COX-2 overexpression, in addition to hypertrophy, cardiac deterioration occurred and was improved by COX-2 inhibitors, suggesting that COX-2 exerts cardiodepressive effects itself.^{17–19} Akt, which may function as an upstream activator or downstream effector of the COX-2 gene, was found to regulate COX-2 transcription in vitro by the increased activity of nuclear factor- κ B, indicating that Akt is an upstream regulator.¹⁵ In contrast, one study on hepatocellular cancer cells described Akt phosphorylation specifically at Thr308 as a downstream signaling event induced by COX-2 overexpression. Phosphorylation of Akt at Thr308 is a prerequisite for activation, whereas phosphorylation of Ser473 solely does not result in full activation.¹⁵

In the present study, there was a significant increase of COX-2, p-Akt (both at Thr308 and Ser473), and p-Erk 1/2 in cardiac tissue before LVAD support compared with controls, and the levels of these parameters were significantly decreased after LVAD support. There are only limited data concerning changes of COX-2 expression on unloading. Razeghi and colleagues²⁰ reported a lack of transcriptional alterations of the COX-2 gene. In their study, none of the

components of the PKB/Akt/GSK3 β pathway were altered by LVAD support, which is in contrast with our finding of a significant Akt decrease after LVAD support.²¹

The decrease of COX-2 protein expression in the present study may be largely explained by the profound reduction of volume/pressure load by LVAD normalizing wall tension, thus altering the conditions that cause compensatory hypertrophy, including COX-2 and prostanoids. In the present study only cardiomyocytes expressing COX-2 were enlarged in CHF, but in contrast with COX-2-negative cardiomyocytes, COX-2-positive cardiomyocytes showed significant hypertrophy regression on unloading. This finding and the correlation between COX-2-positive cardiomyocytes and cardiomyocyte diameters give further emphasis to the hypertrophic effect of COX-2, which can be reversibly regulated by LVADs.

However, patients with LVADs receive acetylsalicylic acid (100 mg) per day from the time of implantation. It must be considered that some of the cellular and molecular changes observed during “reverse remodeling” may be affected by drugs. Aspirin is the only nonsteroidal anti-inflammatory drug that reacts covalently with the COX domain of COX-1 and COX-2, resulting in permanent loss of the COX activity.²² The antithrombotic effect of aspirin is largely caused by the suppression of platelet TX A₂ production by COX-1.²³ However, much higher doses are required to affect COX-2 activity.^{24,25} The concentrations used in vitro are several orders of magnitude higher than the concentrations of aspirin in the serum, which indicates that the changes found in our study are largely the result of LVAD treatment.

Furthermore, the correlations between COX-2 and p-Akt^(Thr308), as well as COX-2 expression and cardiomyocyte diameters before LVAD support, indicate that both proteins are involved in the regulation of cardiomyocyte hypertrophy in CHF.¹⁶ Moreover, these correlations suggest a specific interaction between COX-2 and p-Akt^(Thr308) through a thus unknown cross-talk between these 2 molecules in the progression of hypertrophy during CHF, which might be disrupted by left ventricular support. However, COX-2 and p-Akt^(Thr308) colocalize in a subset of cardiomyocytes before LVAD support, often in the form of direct overlay in the cytoplasm, whereas after LVAD support this colocalization is hardly detectable and controls are completely devoid of it. Thus, a widely distributed direct interaction between the 2 proteins obviously does not occur in every single cardiomyocyte. Akt phosphorylation by application of prostaglandin E₂ analogues through the prostaglandin receptor EP₂ was demonstrated in an intestinal cell line.²⁶ Moreover, COX-2-induced Akt activation through transactivation of epidermal growth factor receptors by prostaglandin E₂ was shown in vitro.²⁷ Possibly in CHF, Akt activation is

induced by similar autocrine or paracrine action of prostaglandins and their receptors in neighboring cardiomyocytes and/or fibroblasts. There was an overlay of p-Akt^(Thr308) and COX-2 in some cardiomyocytes. As a result of lipofuscin removal by Sudan staining beforehand, autofluorescence is unlikely. Perhaps these signals can be regarded as sites of interaction between the 2 proteins, probably mediated by additional molecules.

Conclusions

This study demonstrates a negative regulation of COX-2, p-Akt^(Thr308), p-Akt^(Ser473), and p-Erk 1/2 protein expression and significant cardiac hypertrophy regression by LVAD support. Only COX-2-positive cardiomyocyte diameters were significantly decreased on unloading, suggesting that the COX-2-mediated growth stimulus is confined by the volume/pressure reduction by LVAD support and that the maladaptive hypertrophic response of cardiomyocytes is finally abrogated. Moreover, a significant correlation between both COX-2 and p-Akt^(Thr308), as well as COX-2 and cardiomyocyte hypertrophy, in CHF indicates a thus unknown mechanism of hypertrophic cellular response, which might also be reversibly regulated by LVADs. Of note, COX-2, p-Akt, and p-Erk 1/2 decreased to near normal levels, whereas cardiomyocyte diameters, albeit significantly reduced, remained higher than in controls, suggesting that in contrast with changes on the molecular level, hypertrophy is not fully reversible by LVADs. One of these hypertrophic stimuli might be a molecular cross-talk between COX-2 and p-Akt^(Thr308), probably further mediated by autocrine or paracrine mechanisms of prostaglandins throughout the myocardium. Eventually, activated Akt would then exert widespread influence on molecules involved in cell growth and survival including p70S6-kinase, mTOR, Bad, and the Forkhead family of transcription factors. Given these observations, novel pharmacologic strategies to manipulate both the COX-2 and PI3K/Akt pathway seems desirable in the prevention of cardiac hypertrophy during CHF.

The skillful technical assistance of Ms Dorothe Möllmann and Mr Peter Babioch is highly appreciated.

References

1. van EV, De Windt LJ. Myocyte hypertrophy and apoptosis: a balancing act. *Cardiovasc Res.* 2004;63:487-99.
2. Rose EA, Gelijns AC, Moskowitz AJ, Heitjan DF, Stevenson LW, Dembitsky W, et al. Long-term mechanical left ventricular assistance for end-stage heart failure. *N Engl J Med.* 2001;345:1435-43.
3. Frazier OH, Benedict CR, Radovancevic B, Bick RJ, Capek P, Springer WE, et al. Improved left ventricular function after chronic left ventricular unloading. *Ann Thorac Surg.* 1996;62:675-81.
4. Baba HA, Grabellus F, August C, Plenz G, Takeda A, Tjan TD, et al. Reversal of metallothionein expression is different throughout the human myocardium after prolonged left-ventricular mechanical support. *J Heart Lung Transplant.* 2000;19:668-74.

5. Razeghi P, Taegtmeier H. Activity of the Akt/GSK-3 β pathway in the failing human heart before and after left ventricular assist device support. *Cardiovasc Res*. 2004;61:196-7.
6. Zafeiridis A, Jeevanandam V, Houser SR, Margulies KB. Regression of cellular hypertrophy after left ventricular assist device support. *Circulation*. 1998;98:656-62.
7. Wohlschlaeger J, Schmitz KJ, Schmid C, Schmid KW, Keul P, Takeda A, et al. Reverse remodeling following insertion of left ventricular assist devices (LVAD): a review of the morphological and molecular changes. *Cardiovasc Res*. 2005;68:376-86.
8. Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci U S A*. 2002;99:13926-31.
9. Smith WL, Garavito RM, DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. *J Biol Chem*. 1996;271:33157-160.
10. Schmedtje JF Jr, Ji YS, Liu WL, DuBois RN, Runge MS. Hypoxia induces cyclooxygenase-2 via the NF-kappaB p65 transcription factor in human vascular endothelial cells. *J Biol Chem*. 1997;272:601-8.
11. Torre-Amione G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB, et al. Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. *Circulation*. 1996;93:704-11.
12. Wong SC, Fukuchi M, Melnyk P, Rodger I, Giaid A. Induction of cyclooxygenase-2 and activation of nuclear factor-kappaB in myocardium of patients with congestive heart failure. *Circulation*. 1998;98:100-3.
13. Liu W, Reinmuth N, Stoeltzing O, Parikh AA, Tellez C, Williams S, et al. Cyclooxygenase-2 is up-regulated by interleukin-1 beta in human colorectal cancer cells via multiple signaling pathways. *Cancer Res*. 2003;63:3632-6.
14. Toker A, Cantley LC. Signalling through the lipid products of phosphoinositide-3-OH kinase. *Nature*. 1997;387:673-6.
15. Leng J, Han C, Demetris AJ, Michalopoulos GK, Wu T. Cyclooxygenase-2 promotes hepatocellular carcinoma cell growth through Akt activation: evidence for Akt inhibition in celecoxib-induced apoptosis. *Hepatology*. 2003;38:756-8.
16. Dorn GW, Force T. Protein kinase cascades in the regulation of cardiac hypertrophy. *J Clin Invest*. 2005;115:527-37.
17. LaPointe MC, Mendez M, Leung A, Tao Z, Yang XP. Inhibition of cyclooxygenase-2 improves cardiac function after myocardial infarction in the mouse. *Am J Physiol Heart Circ Physiol*. 2004;286:H1416-24.
18. Zhang Z, Vezza R, Plappert T, McNamara P, Lawson JA, Austin S, et al. COX-2-dependent cardiac failure in Gh/TG transgenic mice. *Circ Res*. 2003;92:1153-61.
19. Delgado RM, III, Nawar MA, Zewail AM, Kar B, Vaughn WK, Wu KK, et al. Cyclooxygenase-2 inhibitor treatment improves left ventricular function and mortality in a murine model of doxorubicin-induced heart failure. *Circulation*. 2004;109:1428-33.
20. Razeghi P, Bruckner BA, Sharma S, Youker KA, Frazier OH, Taegtmeier H. Mechanical unloading of the failing human heart fails to activate the protein kinase B/Akt/glycogen synthase kinase-3 β survival pathway. *Cardiology*. 2003;100:17-22.
21. Baba HA, Stypmann J, Grabellus F, Kirchhof P, Sokoll A, Schafers M, et al. Dynamic regulation of MEK/Erks and Akt/GSK-3 β in human end-stage heart failure after left ventricular mechanical support: myocardial mechanotransduction-sensitivity as a possible molecular mechanism. *Cardiovasc Res*. 2003;59:390-9.
22. Lecomte M, Laneuville O, Ji C, DeWitt DL, Smith WL. Acetylation of human prostaglandin endoperoxide synthase-2 (cyclooxygenase-2) by aspirin. *J Biol Chem*. 1994;269:13207-15.
23. Patrono C, Collier B, Dalen JE, FitzGerald GA, Fuster V, Gent M, et al. Platelet-active drugs: the relationships among dose, effectiveness, and side effects. *Chest*. 2001;119:39S-63S.
24. Tegeder I, Pfeilschifter J, Geisslinger G. Cyclooxygenase-independent actions of cyclooxygenase inhibitors. *FASEB J*. 2001;15:2057-72.
25. Capone ML, Tacconelli S, Sciulli MG, Grana M, Ricciotti E, Minuz P, et al. Clinical pharmacology of platelet, monocyte, and vascular cyclooxygenase inhibition by naproxen and low-dose aspirin in healthy subjects. *Circulation*. 2004;109:1468-71.
26. Tessner TG, Muhale F, Riehl TE, Anant S, Stenson WF. Prostaglandin E2 reduces radiation-induced epithelial apoptosis through a mechanism involving AKT activation and bax translocation. *J Clin Invest*. 2004;114:1676-85.
27. Han C, Wu T. Cyclooxygenase-2-derived prostaglandin E2 promotes human cholangiocarcinoma cell growth and invasion through EP1 receptor-mediated activation of the epidermal growth factor receptor and Akt. *J Biol Chem*. 2005;280:24053-63.