A.E. Schäfler

DISLOCATED AMPLATZER OCCLUDER IN THE LEFT ATRIUM SURGICAL RETRACTION AND PFO CLOSURE

Department of Cardiac Surgery, University of Ulm, Germany

A 42 year old man with recurrent episodes of TIA's underwent heart catheterization and was diagnosed a PFO defect. Insertion of an 18 mm Amplatzer occluder under TEE guidance was performed and additional angiographic evaluation showed complete closure of the defect (Fig. 1). The next day, further assessment with MRI technique revealed a dislocation with a persistent left-right shunt of 9 %, and the patient was referred to the cardiac surgery department (Fig. 2).

Opening of the right atrium displayed the Amplatzer occluder in the left atrium with an atrial septal aneurysma (Fig. 3). After simple removal of the occluder and excision of the aneurysm, a direct closure was performed and the patient was discharged 5 days latter.

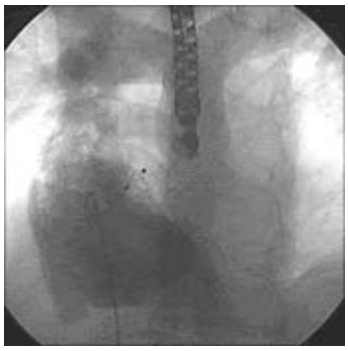

Fig. 1

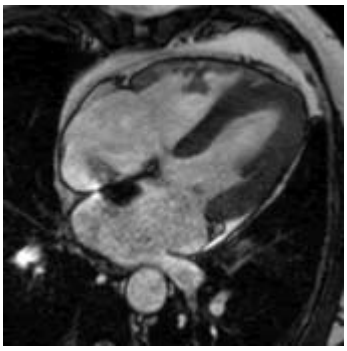

Fig. 2

Fig. 3

e-mail: **aschaefler@gmx.de**

A.E.Schäfler, K.Kirmanoglou

DECREASED ACTIN EXPRESSION IN PATIENTS WITH CHRONIC ATRIAL FIBRILLATION

Department of Cardiac Surgery, University of Ulm, Germany
Department of Surgery, Hospital of Krumbach, Germany

Background - Chronic atrial fibrillation is characterized by a severe contractile dysfunction and myolysis. Remodelling of the cellular ultrastructure develops progressively. Myolysis is associated with the replacement of sarcomeres by glycogen. The aim of our study was, to determine if myolysis is represented by a reduction in actin concentration.

Methods - Right atrial samples from 18 patients undergoing elective cardiac surgery were excised and immediately frozen in liquid nitrogen. 8 patients had chronic AF (≥3 month) and 10 patients were in sinus rhythm (SR). Actin concentration was determined by SDS-PAGE, Western blot and quantified by optical densitometry.

Results - Immunoblot analysis demonstrated actin expression in all hearts. In myocardial samples from patients with chronic AF we found a 2.1 fold reduction in actin expression. ($p<0.001$)

Conclusions - The decrease in actin concentration via myolysis, might decrease energy consumption and be an additional mechanism for contractile dysfunction in chronic AF.

Key Words: atrial fibrillation, heat shock proteins, actin, western blot

1. Introduction

In human atrial fibrillation (AF) [1,2] and pacing induced AF [3,4] substantial changes in atrial myocyte architecture[5,6] and function[7,8] have been reported. Severe myocyte alterations, characterized by enlarged myocytes and myolysis, is observed in fibrillating atria.[9]

Remodelling of the cellular ultrastructure develops progressively. The majority of the cardiomyocytes exhibited marked changes in their cellular substructures, with the replacement of sarcomeres by glycogen as the main characteristic.[10]

The first sign of cellular structural remodeling is a more homogeneous chromatin distribution. Sub-structural changes in mitochondria and sarcoplasmic reticulum occurred gradually. Cellular degeneration was absent. The degree of myolysis and glycogen accumulation increased. Almost half of the myocytes in the right atrial free wall were affected by myolysis. [11]

The aim of this study was to examine if myolysis, with the replacement of sarcomeres by glycogen granulas is rep-

resented by a reduction in actin expression.

2. Material and Methods

2.1 Patients

The present study was reviewed and approved by the ethical committee of the University of Ulm. Analyses of actin levels were performed in myocardium from 18 human hearts. Atrial myocardium was obtained before extracorporeal circulation from 10 patients in sinus rhythm and 8 patients with atrial fibrillation undergoing elective cardiac surgery for coronary revascularisation and/or aortic respective mitral valve replacement. Chronic AF was considered as permanent AF for more than three month.[12] In all patients, except one, CAF was documented with serial ECG's, ultrasound and a history for more than 3 month. The clinical characteristics of the patients are given in Table 1. (Patient 1 through 18): 16 were male and 2 were female; their mean age was 69 ± 9 years. Anesthesia was the same for each patient. Cardiopulmonary bypass was established with a priming solution (1000 ml Ringer, 400 ml human albumin, 200 ml Trasylol, 5000 IU Heparin) at a flow rate of 2,4 l/ min/m² body surface area. All patients were cooled to 32 °C (esophageal). In addition, there was aortic crossclamping, with a myocardial arrest induced by antegrade infusion of cold Brettschneider cardioplegic solution at a myocardial temperature of approximately 10°C.

2.2 Western Blot

2.2.1 Protein Preparation

Myocardium for Western blot analyses was dissected, immediately frozen in liquid nitrogen, and stored at –80°C until use. Care was taken not to use fibrotic or adipose tissue. For protein preparation, » 40 mg of frozen atrial tissue was homogenized by use of a Ultra-Turrax T8 micro dismembrator (IKA Labortechnik, Staufen) for 60 seconds in a nine fold lysis buffer (mmol/L) $Na_4P_2O_7$ 100, EGTA 5, $MgCl_2$ 5, KCl 300, DTT 1, [pH 8,5] along with a protease inhibitor cocktail 0,1 mmol/L AEBSF, containing E-64, bestatin, leupeptin and aprotinin [P2714, Sigma Ltd] at 4°C. The homogenate was centifuged at 4000 rpm for 10 min at 4°C in a Labofuge GL (Heraeus Sepatech). After centrifugation, the supernatant was carefully removed.

Table 1

Patient	Age	Sex	Op	Rhythm
1	65	M	ACVB	SR
2	55	M	ACVB	SR
3	69	M	ACVB	SR
4	78	M	ACVB	SR
5	55	M	ACVB	SR
6	79	M	AVR	SR
7	48	M	ACVB	SR
8	60	M	MVR	SR
9	75	M	ACVB	SR
10	75	F	ACVB	SR
1	74	M	AVR	AF
2	70	M	AVR	AF
3	71	M	MVR/ACVB	AF
4	74	F	MVR	AF
5	69	M	MVR/ACVB	AF
6	78	M	ACVB	AF
7	66	M	ACVB	AF
8	79	M	ACVB	AF

2.2.2 Western Blot Analysis

Samples of 100 µl protein of the particulate fraction were denaturated by heating to 95°C in 900 µl 2 % sodium dodecyl sulfate (SDS), 10 % Glycerol, 5 % 2-mercaptoethanol, 0,002 % bromphenol blue, 0,0625 M Tris-HCL, [pH 6,8] and subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE was performed under reducing conditions on a 10 % separation gel with 4 % stacking gel in a Miniprotean III cell (Bio-Rad Ltd). Running conditions were 200 V at 50 mA for 30 minutes on ice with a PowerPac 3000 (Bio-Rad Ltd). Proteins were transferred to a Hybond-ECL nitrocellulose membrane (Amersham Buchler Ltd) by semidry electroblotting with a Trans blot SD semi dry transfer cell (Bio-Rad) at 5 mA/cm² for 25 min by use of 25 mmol/L Tris, 192 mmol/L glycine, and 20 % methanol [pH 8,3] as a blotting buffer. The transfer was checked by staining the nitrocellulose membrane with 0.1 % Ponceau S solution (Sigma Ltd) in 5 % acetic acid. The blot was blocked 1 hour in 5 % nonfat milk solution (20 mmol/L Tris-HCl, 137 mmol/L NaCl, and 5 % nonfat milk powder, pH 7,45). For immunodetection, the blot was incubated with a 1:10000 diluted mouse anti-human actin monoclonal antibody solution (Clone AC-40, Sigma) for 1 hour (dilution buffer, 20 mmol/L Tris-HCL, 137 mmol/L NaCL, 0,1 % Tween 20, pH 7,45) and washed two times for one minute and three times for five minutes in 10 ml TTBS solution. Immunodetection of the primary antibody against actin was carried out with a 1:4000 diluted peroxidase - conjugated anti mouse secondary antibody (Amersham Ltd) for 60 minutes (dilution buffer, 20 mmol/L Tris-HCL, 137 mmol/L NaCL, 0,1 % Tween 20, pH 7,45). The blot was washed again two times for one minute and three times for 10 min in TTBS, then incubated with 0,125 ml/cm² ECL-detection reagent (Amersham buchler Ltd) for 1 minute, and exposed to Hyperfilm ECL (Amersham Ltd) for 1 minute.

2.2.3 Quantification of Immunoreactive Bands

After development the blots were scanned with a Umax Mirage II densitometer (Umax, Freemont, CA) and a Epson Perfection 1240 Photo (Epson® Europe). Bands were quantified with an analysis software (mars 98, version 1.0.1) according to the densitometric integral derived from each sample band. The integral of the density over the measured area, was taken to calculate the amount in each sample according to the known standard values. A linear relationship was found between the known protein amounts and the densitometric integrals. On the basis of this linear relationship, actin of each atrial sample was calculated ($r = 0.96$).

2.2.4 Statistical analysis

After testing for normal distribution, all data are presented as mean ± SD. To detect differences between groups, the unpaired two tailed Student t-test was performed. The $P < 0,05$ value was considered statistically significant. The correlation between standard HSP60 and the densitometric integral was examined by linear regression analysis.

3. Results

The surgical outcome was uneventful in all patients, and there was no complication from right atrial dissection. The bypass time was approximately 114,6 ± 30 min and the ischemic time 66,7 min ± 21 min.

3.1 Protein expression in myocardium of patients in sinus rhythm and atrial fibrillation

In order to evaluate whether there were any changes in actin expression in permanent atrial fibrillation, Western blot analysis was carried out on SDS-PAGE separations of total protein isolated from ten hearts in SR Fig. [1] and eight hearts in AF Fig. [2].

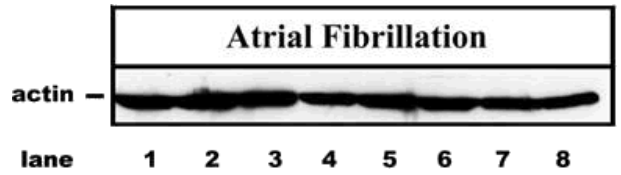

Fig. 1

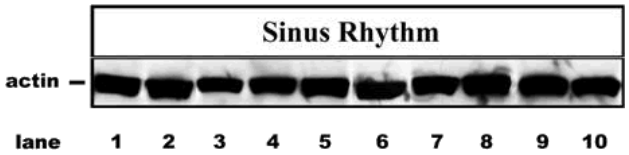

Fig. 2

The following values refer to a concentration of nanogram protein per milligram wet heart tissue. In patients with chronic AF there was a 2.06 fold decrease (3052 ng ± 858 ng) in actin concentration compared to (6278 ng ± 1354 ng; * $p < 0.001$) in patients with SR.

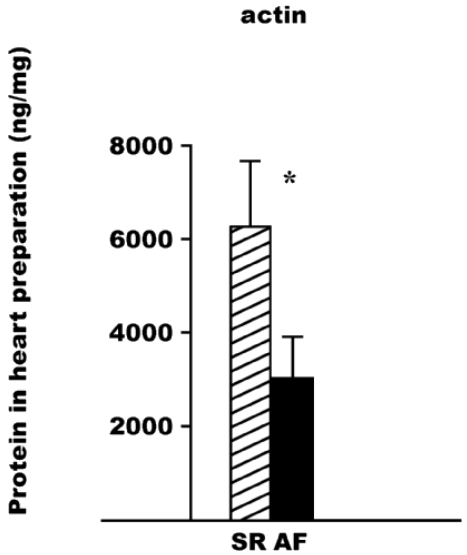

Fig. 3

4. Discussion

We investigated the influence of the chronic increased atrial activity on actin expression in right atrial appendages. The present study demonstrates that myocardium from chronic fibrillating right atrial appendages shows a more than 2-fold decrease in actin concentration.

Microscopically, this is reflected through the replacement of sarcomeres by glycogen granulas.[13] On the protein level the decrease in contractile elements is represented by a decrease in actin concentration.

Short episodes of AF with increased atrial activity lead to an imbalance in energy supply. AF was associated with a transient lowering of phosphocreatine content, suggesting an increase in energy demand during the early phase of AF.[14] This may cause functional impairment with a subsequent decrease in atrial transport function.[15] To counteract the decrease in energy equivalences, short term pacing induced AF significantly activated membrane F0F1-ATPase activity.[16] The subsequent recovery of the phosphocreatine pool indicates restoration of the balance between energy demand and supply in chronic fibrillating atria.[14]

In chronic AF with enhanced cellular metabolism strategies are necessary to reduce energy consumption. This signal leads to a change in the transcription program and cellular reorganization is accomplished.

The reduction in contractile elements will decrease force development and energy consumption, while the storage of high energy equivalences will ensure supply. The decrease in actin expression, together with the functional decrease in force in the atrial trabecula system, may represent an additional mechanistic basis for the contractile impairement observed in these patients.

In summery this investigation shows an additional adaptive response to chronic AF and provides new insight regarding atrial subcellular structure in chronic AF.

References

1. Connelly JH, Clubb FJ, Vaughn W, Duncan M. Morphological changes in atrial appendages removed during the maze procedure: a comparison with autopsy controls. Cardiovasc Pathol 2001;10:39-42.
2. Ad N, Snir E, Vidne BA, Golomb E. Histologic atrial myolysis is associated with atrial fibrillation after cardiac operation. Ann Thorac Surg. 200;72:688-93.
3. Everett TH 4th, Li H, Mangrum JM, McRury ID, Mitchell MA, Redick JA et al. Electrical, morphological, and ultrastructural remodeling and reverse remodeling in a canine model of chronic atrial fibrillation. Circulation. 2000;102:1454-60.
4. Ausma J, Dispersyn GD, Duimel H, Thone F, Ver Donck L, Allessie MA et al. Changes in ultrastructural calcium distribution in goat atria during atrial fibrillation. J Mol Cell Cardiol. 2000;32:355-64.
5. Shirani J, Alaeddini J. Structural remodeling of the left atrial appendage in patients with chronic non-valvular atrial fibrillation: Implications for thrombus formation, systemic embolism, and assessment by transesophageal echocardiography. Cardiovasc Pathol. 2000;9:95-101.
6. Kostin S, Klein G, Szalay Z, Hein S, Bauer EP, Schaper J. Structural correlate of atrial fibrillation in human patients. Cardiovasc Res 2002;54:361-79.
7. Schotten U, Ausma J, Stellbrink C, Sabatschus I, Vogel M, Frechen D, Schoendube F, Hanrath P, Allessie MA. et al. Cellular mechanisms of depressed atrial contractility in patients with chronic atrial fibrillation. Circulation 2001;103:691-98.
8. Sun H, Gaspo R, Leblanc N, Nattel S. , et al. Cellular Mechanisms of Atrial Contractile Dysfunction Caused by Sustained Atrial Tachycardia. Circulation. 1998;98:719-27.
9. Rucker-Martin C, Pecker F, Godreau D, Hatem SN. Dedifferentiation of atrial myocytes during atrial fibrillation: role of fibroblast proliferation in vitro. Cardiovasc Res. 2002 Jul;55(1):38-52.
10. Ausma J, Wijffels M, van Eys G, Koide M, Ramaekers F, Allessie M, Borgers M. Dedifferentiation of atrial

cardiomyocytes as a result of chronic atrial fibrillation. Am J Pathol. 1997 Oct;151(4):985-97.
11. Ausma J, Litjens N, Lenders MH, Duimel H, Mast F, Wouters L, Ramaekers F, Allessie M, Borgers M. Time course of atrial fibrillation-induced cellular structural remodeling in atria of the goat. J Mol Cell Cardiol. 2001 Dec;33(12):2083-94.
12. Sopher SM, Camm AJ. Atrial fibrillation: maintenance of sinus rhythm versus rate control. Am J Cardiol. 1996;77:24A-37. A review
13. Thijssen VL, Ausma J, Liu GS, Allessie MA, van Eys GJ, Borgers M. Structural changes of atrial myocardium during chronic atrial fibrillation. Cardiovasc Pathol. 2000 Jan-Feb;9(1):17-28. Review.
14. Ausma J, Coumans WA, Duimel H, Van der Vusse GJ, Allessie MA, Borgers M. Atrial high energy phosphate content and mitochondrial enzyme activity during chronic atrial fibrillation. Cardiovasc Res. 2000;47 (4):788-96.
15. Daoud EG, Marcovitz P, Knight BP, et al. Short-Term Effect of Atrial Fibrillation on Atrial Contractile Function in Humans. Circulation 1999;99:3024-27.
16. Barbey O, Pierre S, Duran MJ, Sennoune S, Levy S, Maixent JMJ. Specific up-regulation of mitochondrial F0F1-ATPase activity after short episodes of atrial fibrillation in sheep. Cardiovasc Electrophysiol 2000;11 (4):432-8

e-mail: **aschaefler@gmx.de**

A.E. Schäfler. Ph.Weigold

SERUM ANTIBODIES TO HUMAN HEAT SHOCK PROTEIN 70 IN PATIENTS WITH CORONARY ATHEROSCLEROSIS

Department of Cardiac Surgery, University of Ulm, Germany
Department of Surgery, Hospital of Krumbach, Germany

Abstract: Previous studies suggest that antibodies to human heat shock protein 70 have a higher frequency in patients with risk factors for coronary atherosclerosis. We examined whether circulating anti-human heat shock protein 70 antibodies are associated with coronary artery disease. In a case control study preoperative blood samples for immunoblot analyses from 117 patients with severe coronary artery disease and 50 patients without coronary atherosclerosis were tested. Serum heat shock protein 70 antibodies were detectable in 8 patients undergoing bypass operations and one patient with aortic valve replacement. No association between anti-heat shock protein 70 IgG seropositivity and the prevalence of coronary artery disease was found (p = 0.28). These data provide evidence that anti human heat shock protein 70 IgG antibodies are not associated with coronary atherosclerosis.
Key words: heat shock protein 70, antibody, coronary atherosclerosis, cardiac surgery

1. Introduction

Autoantibodies against certain stress or heat shock proteins (HSP) may play a role in the pathogenesis of atherosclerosis.[1] Heat shock proteins are components of a physiologic stress response and Hsp70 is a highly conserved immunogenic molecule.[2] Many reports indicate that antibodies against HSPs are present in human serum.[3,4,5]

In patients with risk factors for coronary atherosclerosis a higher frequency of antibodies against human HSP70 are documented. There was a statistical association of antibodies against Hsp70 with hypertension[6] and hsp72 antibodies could be detected more frequently in smokers.[7] Additionally HSP 70 antibodies were more often present among Type 1 diabetes subjects.[8,9]

Therefore we tested the hypothesis if patients with severe coronary atherosclerosis undergoing CABG surgery, have a higher frequency of anti human HSP70 IgG antibodies.

2. Material and methods

2.1 Patients

The present study was reviewed and approved by the ethical committee of the University of Ulm. Analyses of antibody levels against human HSP70 were performed in serum from 167 subjects. Serum was obtained the day of admission from all patients undergoing elective cardiac surgery. The characteristics are given in Table 1.

2.2 Immunoblot Analysis

2.2.1 Serum Preparation

Circulating antibodies were determined in serum samples obtained from all study subjects after centrifugation at 4000 rpm for 4 min in a Labofuge GL (Heraeus Sepatech, Biofuge primo R) and stored at 4 °C.

Table 1.

Characteristics of the study subjects. ACB indicates aorto-coronary bypass; MVR mitral valve replacement/repair; AVR aortic valve replacement/repair; m male; f female; y years; m male; prot. protein; conc. concentration; mg milligram; ml millilitre;

Group	total n	ACB n	AVR/ MVR n
Patients	167	117	50
Age [y]	59.0 ± 18.4	65.7 ± 8.6	70.4 ± 9.1
sex [m/f]	119/48	93/24	26/24
prot. conc. [mg/ml]	31.3	32.6	30.1
anti-hHSP70	9	8	1