Pathophysiological findings in a model of persistent atrial fibrillation and severe congestive heart failure

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Abstract

Objective: Develop and evaluate a model of persistent atrial fibrillation (AF) and severe congestive heart failure (CHF). Methods: A single-chamber atrial pacemaker was implanted in pigs (20–30 kg). Burst atrial pacing at 42 Hz led to development of persistent AF. Immediately and 20 days after activation of the burst pacing protocol, animals underwent echocardiography. Heart rate, rhythm and general condition were monitored on a daily basis. After 20 days of atrial fibrillation, the animals were sacrificed. Conventional histological methods were used to evaluate microscopic structural changes. Results: In the pig model, persistent atrial fibrillation developed 5 ± 0.7 days after initiation of the burst protocol. Ventricular response rate was 274 ± 5 bpm during atrial fibrillation, leading to a tachycardiomyopathy. Heart failure symptoms occurred approximately 15 days after initiation of burst pacing. Increases in QT interval on electrocardiogram, heart weight-to-body weight ratio, and laboratory values suggestive of a hypercatecholaminergic state, as well as liver and kidney dysfunction occurred during the 20-day duration of the study. Microstructural changes consistent with cellular hypertrophy, variable fibrosis, myolysis and apoptosis were found in the atria and ventricles of the study animals. Conclusions: The combined entity of atrial fibrillation and severe congestive heart failure leads to multiple organ dysfunction, ultrastructural and microscopic cardiac changes. Cellular hypertrophy, fibrosis and apoptosis are more prominent in this combined entity than previously reported models of lone atrial fibrillation or heart failure. This model can be used for further investigation into the pathophysiology and treatment of atrial fibrillation and advanced heart failure. © 2004 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: Atrial fibrillation; Congestive heart failure; Cardiac hypertrophy; Cardiac dilation; Apoptosis

1. Introduction

The coexistence of atrial fibrillation (AF) and congestive heart failure (CHF) is an all too common clinical situation. Both CHF and AF are causally diverse and multifactorial, but similarities in risk factors are described [2]. The situation is considerably more complex, however, because the two conditions seem to feedback upon each other. People with AF are predisposed to developing CHF [2], and the incidence of AF increases as the heart failure worsens [1]. Both diagnoses are responsible for a dramatic impact on patient lives, and the two together portend a significantly worse prognosis than would be expected when considering the two diagnoses individually [2]. Within this complexity is an increased risk for stroke, sudden death and pump failure death. As yet, the interaction between CHF and AF is poorly understood.

Treatment options for chronic AF in CHF are limited. The utility of antiarrhythmic drugs is limited by proarrhythmia and inefficacy [2]. Rate control is often difficult because effective doses of rate-controlling drugs (beta- and Ca-channel blockers) are generally not tolerated in the CHF population [2]. As a result, digoxin is often used as the primary rate-controlling agent, with variable efficacy. Compounding this problem is the lack of an experimental model to study the combined entity of chronic AF and severe CHF. A burst atrial pacing model of chronic AF in goats does not have significant CHF [3], and a ventricular-pacing model of CHF has only been tested with acute, self-limited rather than persistent AF [4].

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Here, we describe a porcine model of persistent AF with severe CHF caused by the uncontrolled ventricular response to the AF. The severity of the CHF and the interaction with chronic AF are novel observations. This model will be useful to investigate the pathophysiology and possible treatment options of the combined entity of chronic AF and severe CHF.

2. Methods

2.1. Experimental preparation

In total, 20 domestic swine (20–30 kg, 3–5 months of age) were used in this study. Five served as a control group with pacemaker implantation, but without burst atrial pacing. Fifteen animals underwent the following burst pacing protocol: prior to pacemaker implantation, pigs were anaesthetized with ketamine (50 mg/kg) and sodium pentothal (20 mg/kg) and ventilated with isoflurane (0.5–2%) and oxygen through an endotracheal tube. Under sterile conditions, a bipolar active-fixation pacing lead (Medtronic, Minneapolis, USA) was inserted via the right external jugular vein and fixed in the right atrial appendage under fluoroscopic guidance. The lead was connected to the pacemaker (Medtronic Thera), and the pacemaker unit was positioned in a subcutaneous pocket in the neck. The pocket was perfused with 500 ml of biobiotic solution (neomycin 40 mg, polymyxin B 2 HTU) to prevent infection. Sixty minutes after closure of the incision site, the pacemaker was programmed to a sinus rhythm detection rate of 180/min, and a pacing frequency of 42 Hz.

Clinical observations and ECG recordings were performed using a six-lead ECG system that was attached to the animals while they were feeding. In this way, the animals were fully awake and alert, but they were also calm and cooperative. Electrodes position was reproducibly obtained by placement on tattoo marks placed at the time of pacemaker implantation. QT and RR intervals were measured in 25 consecutive beats, and the average values were used. The measurements were taken using a six-lead ECG system, and the QT interval was defined as the time from the start of the QRS complex to the end of the T wave in all leads. For comparative QT measurements, recordings were compared from nonsustained AF during burst pacing post-implant on day 0 to those from presacrifice on day 25. As such, heart rates were comparable for the two measurements. For further heart rate correction, the corrected QT interval (QTc) was calculated using Bazett’s formula. For calculations of growth rate and the heart weight–body weight ratio (HW/BW ratio) pigs were weighed on a weekly basis. Heart weights were determined on the day of euthanization. Blood samples were taken at the time of sacrifice, and analysis of electrolytes, metabolic parameters and serum catecholamine levels was performed by Quest Diagnostics (Baltimore, MD).

The animals for this study were maintained in accordance with the guiding principles of the American Physiological Society regarding experimental animals. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the Johns Hopkins University, and the study design conforms to NIH guidelines for animal use.

2.2. Echocardiographic examinations

Echocardiographic examinations were performed on the day of pacemaker implantation (day 0) and 20 days thereafter. For consistency, EF and chamber sizes were evaluated during paroxysmal AF in the first study and during persistent AF on day 20. In that way, heart rate and rhythm were similar for the two studies. Left ventricular ejection fractions (LVEF) were calculated from parasternal short- and long-axis views using the software provided with the system (Agilent 5500, MA, USA). Chamber sizes were determined from M mode images. All measurements were made using the American Society of Echocardiography criteria [5].

2.3. Histological evaluation

After euthanization of animals, hearts were removed and rinsed thoroughly in PBS. Sections of atria and ventricles were dissected from the heart, rapidly frozen in dry ice-cooled ethanol and stored at −80 °C. To avoid any confounding factors related to the pacemaker lead, right atrial sections were taken from the anterior-lateral atrial wall, away from the lead tip and from any fibrotic adhesions between the lead body and the heart. The sections for microscopic analysis were fixed in 10% formalin, embedded in paraffin, cut to 7-μm thickness and stained with haematoxylin and eosin or Masson’s trichrome using traditional methods. Masson’s trichrome staining was used to identify increased concentration of interstitial fibrosis.

Samples were examined at 64 × magnification in a random-order and blinded fashion by two observers. For each reviewer, all samples were evaluated in one sitting, using the same microscope at the same magnification. Samples from each chamber (LA, RA, LV, RV) were considered separately. The reported histological score is the average of these observations. Variables included nuclear enlargement, cellular hypertrophy, cellular myolysis, interstitial fibrosis and interstitial inflammation. Each sample was graded on a scale of 1–5 based on the extent of a given observation throughout the sample. In general, a score of 1 signified normal; 2 indicated that the observation in question was occasionally present (i.e., occasionally notable nuclear enlargement with mostly normal nuclear size); a score of 3 was given for diffuse abnormalities encompassing approximately half of the cells or tissue; 4 was given if the
observation in question was present diffusely throughout the tissue; and a score of 5 indicated near complete presence of the variable under examination.

2.4. TUNEL staining

Tissue specimens were embedded in paraffin and cut to 7-μm thickness. Then, tissue was dewaxed and rehydrated according to standard protocols [6]. Preparation of tissue for TUNEL staining was performed following the protocol provided by Roche Applied Science. Tissue was placed on glass slides and incubated in a plastic jar containing 200 ml 0.1 M citrate buffer (Sigma Aldrich) and 0.1% Triton (Roche Applied Science, Mannheim, Germany), pH 6.0. The sections were applied to 350 W microwave irradiation for 5 min, cooled rapidly by adding 80 ml distilled water (20–25 °C) and rinsed with PBS at 15–25 °C. Afterwards 50 μl of TUNEL reaction mixture (Roche Applied Science) was added to the sections, and glass slides were incubated for 60 min at 37 °C. Finally, slides were rinsed three times in PBS. Negative controls were sections incubated with label solution alone, and sections incubated with DNase I (Sigma Aldrich) served as positive controls. Following the staining procedure, samples were analyzed for TUNEL-positive cells using a fluorescent microscope. The percentage of TUNEL-positive cells in each chamber was calculated by dividing the number of green cells by the total number of cells in randomly selected high-power fields and multiplying by 100%.

2.5. Statistical analyses

The data are presented as mean ± S.E.M. Statistical significance was determined at the 5% level using the Student’s t-test and repeated-measures ANOVA, where appropriate.

3. Results

3.1. Clinical observations during induction of persistent AF and CHF

The five control pigs maintained sinus rhythm and had unremarkable clinical courses for the duration of the study. The 15 animals undergoing burst atrial pacing initially had 2–10-s increments of AF following each burst of atrial pacing, and they were in persistent AF 5 ± 0.7 days (range 3–11 days) after initiation of the burst pacing protocol. On days 1–5 of self-limited AF, the ventricular response rate was 274 ± 3 bpm during the combined period of burst pacing and nonsustained AF, and the sinus rate was 115 ± 17 bpm. During the period of sustained AF, the average ventricular response during waking hours was 270 ± 5 bpm, not significantly different than the ventricular response during the burst pacing and nonsustained AF on days 1–5 (Fig. 1).

Over the first 15 days of the study, the control and AF/CHF animals were indistinguishable in behavior, activity and eating patterns. Between days 15–20, the AF animals moved more slowly from their cages to the ECG recording and feeding area. They also had decreased appetites and decreased activity inside their cages. These symptoms varied, with some AF pigs appearing lethargic 1 day and normal the next. The average weekly weight gain was 9.7 ± 0.8% for the AF/CHF group and 9.0 ± 1.0% for the controls (p = NS). Over the duration of the study, no ventricular arrhythmias, sudden deaths, or strokes were observed. At the time of sacrifice, no blood clots were observed in the atria of any animals.

At the conclusion of the study, 20 days after initiation of the burst pacing protocol, the QT measurements were repeated. To allow comparison at similar heart rate and regularity, QT measurements taken initially during nonsustained AF on day 0 were compared to those in persistent AF at similar ventricular response rates on day 20. Compared to measurement on day 0, there was a significant increase in QTc interval (400 ± 3 ms, day 0 vs. 470 ± 3 ms, day 20, p = 0.0001). In contrast to the changes seen in the heart failure group, control animals without AF or CHF had no significant change in QT interval over a 20-day observation period (405 ± 5 day 0 vs. 398 ± 3 day 20, p = NS).

At the conclusion of the study, all animals had blood taken for chemical analysis (Table 1). Significant differences were found in cardiac and liver enzymes, kidney function, total protein and serum albumin. The heart failure animals also had significant elevations in circulating noradrenaline levels. After sacrifice, the AF/CHF animals were noted to have significant ascites and negligible body fat, suggesting that the observed weight gain in the AF/CHF...
CHF group resulted from water accumulation not growth. Neither of these findings was noted in the control animals. Heart weight-to-body weight (HW/BW) ratio was markedly elevated in the AF/CHF group (7.3 \pm 0.4 vs. 4.9 \pm 0.6 g/kg control, \( p = 0.001 \)).

### 3.2. Ultrastructural changes in animals with AF/CHF

Echocardiographic measurements from day 0, during burst atrial pacing, were compared to those on day 20 during persistent AF. Mean heart rate was not significantly different during the two echocardiographic studies (day 1: 269 \pm 8 bpm, day 20: 272 \pm 7 bpm, \( p = \text{NS} \)), so the measurements were comparable. The AF/CHF animals had significant increases in left atrial diameter, left ventricular end-systolic and end-diastolic diameter and left ventricular wall thickness (Table 2). Left ventricular ejection fraction (LVEF) was markedly diminished on day 20 (Fig. 2A). No similar changes were observed in the control group. In the AF/CHF animals, change in LVEF correlated with average ventricular rate during the 20-day study duration (Fig. 2B), although there was no correlation between heart rate and either atrial or ventricular chamber size.

### 3.3. Microscopic changes in atria and ventricles

Both control and failing hearts were subjected to routine histological analysis using hematoxylin and eosin and Masson’s trichrome-stained sections. Figs. 3 and 4 illustrate some of the pertinent pathological differences between control and failing hearts. In both LV and RV, signs of hypertrophy, interstitial inflammation and myolysis were evident after 20 days of AF. Atrial images from AF/CHF pigs showed an increase in nuclear and total cell volume and an increased amount of interstitial inflammatory cells and myolysis. These findings were more prominent in the right atrium (the side with the pacing lead) than in the left atrium. Comparative images from control animals demonstrate normal tissue architecture.

Masson’s trichrome staining was used to evaluate the extent of fibrosis in all tissue sections. Fibrosis generally appears in two forms, reparative and reactive. Reparative fibrosis is mainly interstitial, and reactive fibrosis is primarily perivascular. Both reparative and reactive fibrosis were evident in all chambers of AF/CHF pigs. Left ventricular images from failing hearts demonstrated a large amount of fibrosis, whereas control pigs showed minimal fibrous tissue. In contrast, the RV sections from AF/CHF animals had significantly less fibrosis than LV sections and were not significantly different than controls. Right atrial images from failing hearts demonstrated a large amount of fibrosis.

### Table 1

<table>
<thead>
<tr>
<th>Blood chemistry values in normal and AF/CHF animals</th>
<th>Control</th>
<th>AF/CHF</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>1.2 \pm 0.2</td>
<td>2.2 \pm 0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BUN</td>
<td>13 \pm 3</td>
<td>23 \pm 2</td>
<td>0.01</td>
</tr>
<tr>
<td>Protein</td>
<td>6.2 \pm 0.2</td>
<td>5.3 \pm 0.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.5 \pm 0.1</td>
<td>2.9 \pm 0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>92 \pm 14</td>
<td>92 \pm 4</td>
<td>NS</td>
</tr>
<tr>
<td>Alkaline phosphate</td>
<td>189 \pm 35</td>
<td>175 \pm 5</td>
<td>NS</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.1 \pm 0</td>
<td>0.1 \pm 0</td>
<td>NS</td>
</tr>
<tr>
<td>ALT</td>
<td>38 \pm 4</td>
<td>38 \pm 3</td>
<td>NS</td>
</tr>
<tr>
<td>AST</td>
<td>31 \pm 4</td>
<td>60 \pm 13</td>
<td>0.05</td>
</tr>
<tr>
<td>GGT</td>
<td>24 \pm 4</td>
<td>27 \pm 5</td>
<td>NS</td>
</tr>
<tr>
<td>CPK</td>
<td>821 \pm 90</td>
<td>4231 \pm 1225</td>
<td>0.01</td>
</tr>
<tr>
<td>NE</td>
<td>68 \pm 21</td>
<td>4312 \pm 2911</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Abbreviations: BUN: blood urea nitrogen; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyltransferase; CPK: creatine phosphokinase; NE: norepinephrine.

### Table 2

<table>
<thead>
<tr>
<th>Echocardiographic measurements</th>
<th>Baseline</th>
<th>AF/CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA diameter (cm)</td>
<td>1.5 \pm 0.2</td>
<td>2.9 \pm 0.4*</td>
</tr>
<tr>
<td>RV end-diastolic diameter (cm)</td>
<td>1.9 \pm 0.5</td>
<td>2.7 \pm 0.4*</td>
</tr>
<tr>
<td>LV end-diastolic diameter (cm)</td>
<td>2.6 \pm 0.6</td>
<td>3.8 \pm 0.7*</td>
</tr>
<tr>
<td>LV septum thickness (cm)</td>
<td>0.6 \pm 0.1</td>
<td>0.8 \pm 0.1*</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>70 \pm 2</td>
<td>28 \pm 2*</td>
</tr>
</tbody>
</table>

* \( p < 0.05 \).
whereas control pigs showed minimal fibrous tissue. The left atrial images of both control and AF/CHF animals showed more fibrosis than right atrial images. Relative to the control animals, there was only a nonsignificant trend toward more left atrial fibrosis in the AF/CHF group.

We used TUNEL staining to evaluate the percentage of cells undergoing the apoptotic process (Fig. 5). Positive TUNEL staining indicates DNA damage, a marker of either apoptosis or necrosis. Generally, the two processes can be distinguished by the absence of necrotic signs on micro-

Fig. 3. Histological changes in atrial tissue after 20 days of AF/CHF. (A) Summary of changes in left and right atria compared to controls ($n=5$ for control, 15 for AF, $*p<0.05$, $*p<0.01$). (B) Examples of control and AF/CHF left atria. (C) Examples of control and AF/CHF right atria (magnification 64×).

Fig. 4. Histological changes in ventricular tissue after 20 days of AF/CHF. (A) Summary of changes in left and right ventricles compared to controls ($n=5$ for control, 15 for AF, $*p<0.01$). (B) Examples of control and AF/CHF left ventricles. (C) Examples of control and AF/CHF right ventricles (magnification 64×).
sections of apoptotic cells [7]. Tissue sections from AF/CHF hearts showed no evidence of necrosis (such as contraction bands, coagulation or nuclear pyknosis), suggesting that TUNEL-positive cells were indeed apoptotic. In the AF/CHF group, apoptotic cells were diffusely present (LA 25 ± 5%, RA 19 ± 3%, LV 32 ± 4%, RV 37 ± 6%). In contrast, the control hearts only had rare TUNEL-positive cells. Interestingly, with this AF/CHF model, the percentage of TUNEL-positive cells was markedly higher in the ventricles (35 ± 3%) than in the atria (22 ± 3%, p = 0.01).

4. Discussion

Atrial fibrillation increases morbidity and mortality in congestive heart failure. Loss of the atrial contribution to cardiac output, increased stroke risk, rapid and irregular cardiac contraction and side effects from antiarrhythmic and rate-controlling medications are all strong contributors to this increased risk [2]. Understanding of the physiology of the combined entity of AF and CHF has been limited by the absence of a viable animal model. A model of chronic AF in goats is more closely related to lone AF, because it does not have a heart failure component [8]. A pacing-induced heart failure model in dogs has been shown to have increased susceptibility to induction of AF, but chronic AF is not present in this model [4]. This report is the first documentation of persistent AF in a porcine model. Unlike other animal models, the sustained rapid ventricular response in the pig model causes severe CHF, allowing study of the combined entity of persistent AF and severe CHF. Of note, the animals in our study were juvenile, which likely accounts for their ability to sustain such rapid heart rates during AF. The age of these animals should be kept in mind, because it might affect other physiological observations, which is a limitation to the model that requires further study to define.

4.1. Clinical observations

Signs of heart failure were not apparent during the first 15 days of AF. Over the last 5 days of the study, increasing dyspnea with exertion, reduced activity and appetite were present. Hemodynamic changes over this time period are of interest, but we were unfortunately not equipped to make these measurements. At sacrifice, cachexia and ascites were noted; norepinephrine levels were elevated over 50-fold above controls, and evidence of liver and kidney dysfunction was present. QT prolongation also developed over this time period, which was not noted in the control group. Although QTc was calculated using Bazett’s correction, the heart rates in the AF/CHF group were similar at the time of both measurements, making the observed changes in QT more reliable. All of these observations are consistent with previous reports of human heart failure. Like other animal models, the time course in our model is accelerated out of necessity. The human condition develops over several years, which is not possible in a laboratory environment. That limitation notwithstanding, the cardiac and non-cardiac manifestations of this AF/CHF model closely emulate those reported for the human condition.

4.2. Histological changes in AF/CHF

In the goat model of lone AF, key histological findings include degradation of the myocyte sarcomeric structure (myolysis), glycogen accumulation and cellular hypertrophy [9]. A dog model of ventricular pacing-induced CHF and “increased susceptibility” to AF also had signs of atrial myocyte hypertrophy and myolysis, but also had prominent interstitial fibrosis [4]. Data from human tissue derived from patients with AF and mitral valve disease showed variable fibrosis and prominent hypertrophy and myolysis [7]. These data suggest that AF and CHF independently cause similar alterations in the cellular and tissue structure of the atria, a hypothesis supported by Li et al.’s [4] demonstration of increased susceptibility to AF in dogs with CHF.

Our data extend these findings by showing almost uniform myolysis, but less prominent hypertrophy and fibrosis in pigs with chronic AF and severe CHF. Both reactive and reparative fibrosis are noted in our AF/CHF animals. One potential reason for the variability in fibrosis is the age of the animals. Another important possibility is the short duration of AF/CHF. It is possible that a longer observation period would have shown increased levels of fibrosis. If so, this data would suggest that the cellular changes precede the tissue changes, rather than both happening concurrently. Such information could have implica-
3. Apoptosis in AF/CHF

Prior data on apoptosis in AF is minimal. Dispersyn et al. [10] aggressively searched for signs of apoptosis in the lone AF goat model and found none. Aime-sempe et al. [7] observed TUNEL and CASP-3 positive staining in 4% of cells from atrial tissue samples from AF patients undergoing valve surgery. The extent of heart failure was variable in their study, and they observed that the strongest CASP-3 staining occurred in the most diseased hearts. Our animal model supports this conclusion. The extent of CHF in the pig AF/CHF model is severe, with extensive symptoms as well as physical signs of cardiac enlargement, decreased LVEF and fluid retention. In our model, the degree of apoptosis was extensive in both atria and ventricles. Given the short observation period of our study, two questions arise from this observation. Will the extensive cell death stabilize over subsequent weeks and will it lead to fibroblast infiltration of the tissue and replacement with extensive fibrosis? Further investigation into the time course of this model is warranted, but the current study gives valuable insight into the early stages of persistent AF and CHF.

5. Conclusions

The need for a viable AF/CHF model is illustrated by the differences between human pathology and the observations in the existing animal models. Our new model offers the ability to investigate the interaction between AF and CHF on a variety of different levels. Tolerance of therapeutic interventions can be tested, and the effects on morbidity and mortality can be observed. Ultrastructural changes in structure and function can be observed and traced back to alterations on a cellular and subcellular level. In this report, we demonstrate dilation of all cardiac chambers and reduction in contractile function 3 weeks after the onset of AF with uncontrolled ventricular response rate. Histological changes corresponding to these ultrastructural effects include extensive myolysis, variable fibrosis with the greatest effects seen in the ventricles, cellular hypertrophy and apoptosis. Unlike other animal models, these extensive cellular changes are observed in both atria and ventricles. Further investigation into the time course of these changes has the potential to offer valuable insights into the prevention and treatment of chronic AF in the CHF patient.

Acknowledgements

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