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Heart failure duration progressively modulates the arrhythmia substrate through structural and electrical remodeling

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ABSTRACT

Aims: Ventricular arrhythmias are a common cause of death in patients with heart failure (HF). Structural and electrical abnormalities in the heart provide a substrate for such arrhythmias. Canine tachypacing-induced HF models of 4–6 weeks duration are often used to study pathophysiology and therapies for HF. We hypothesized that a chronic canine model of HF would result in greater electrical and structural remodeling than a short term model, leading to a more arrhythmogenic substrate.

Main methods: HF was induced by ventricular tachypacing for one (short-term) or four (chronic) months to study remodeling.

Key findings: Left ventricular contractility was progressively reduced, while ventricular hypertrophy and interstitial fibrosis were evident at 4 month but not 1 month of HF. Left ventricular myocyte action potentials were prolonged after 4 ($p < 0.05$) but not 1 month of HF. Repolarization instability and early afterdepolarizations were evident only after 4 months of HF ($p < 0.05$), coinciding with a prolonged QTc interval ($p < 0.05$). The transient outward potassium current was reduced in both HF groups ($p < 0.05$). The outward component of the inward rectifier potassium current was reduced only in the 4 month HF group ($p < 0.05$). The delayed rectifier potassium currents were reduced in 4 ($p < 0.05$) but not 1 month of HF. Reactive oxygen species were increased at both 1 and 4 months of HF ($p < 0.05$).

Significance: Reduced I_{to}, outward I_{K1}, I_{Ks}, and I_{Kr} in HF contribute to EAD formation. Chronic, but not short term canine HF, results in the altered electrophysiology and repolarization instability characteristic of end-stage human HF.

Introduction

Heart failure (HF) is a leading contributor to morbidity and mortality. In 2010, the number of deaths in the US attributed to HF was ~279,000, and HF was noted in 1 of 9 death certificates [1]. Sudden death due to lethal ventricular arrhythmias is six- to nine-fold higher in HF patients than in the general population [2,3], and accounts for up to 50% of deaths in HF patients [2].

HF is associated with both structural and electrical remodeling that transforms the normal myocardium into a substrate susceptible to arrhythmogenesis. Left ventricular dilation, hypertrophy, and fibrosis are all examples of compensatory changes that are initially adaptive in the failing heart [4]. These changes may progress to become maladaptive resulting in further deterioration of heart function, and have been linked to the development of arrhythmia and/or sudden death [5–7]. At the cellular level, cardiomyocytes of the failing heart display electrical remodeling, including a signature prolongation of the action potential (AP) [8,9].

We previously reported that chronic (four or more months) canine tachypacing HF becomes irreversible and emulates multiple aspects of chronic human HF [10]. In the present study, we tested the hypothesis

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Table 1
Echocardiographic and electrocardiogram parameters in 4 M HF animals.

	Baseline	1 month HF	4 month HF
Fractional shortening (%)	29.6 ± 1.49	15.2 ± 0.97*	11.2 ± 0.84*,**
Left ventricle dimension (cm)			
Diastole	3.46 ± 0.13	4.33 ± 0.15*	5.28 ± 0.21*,**
Systole	2.41 ± 0.07	3.67 ± 0.12*	4.68 ± 0.18*,**
Left ventricle mass (g)	91.6 ± 9.15	121.2 ± 11.0	157.7 ± 16.1*,**
ECG parameters			
PR (ms)	110.7 ± 5.24	108.1 ± 5.01	107.9 ± 3.85
QRS (ms)	45.8 ± 2.47	50.1 ± 3.03	54.2 ± 4.10*
RR (ms)	578.4 ± 35.4	520.2 ± 53.3	511.2 ± 69.4
QT (ms)	201.1 ± 6.10	199.6 ± 7.40	198.9 ± 8.38
QT_{cf} (ms)	242.5 ± 3.35	250.1 ± 4.59	252.4 ± 4.33*

N = 7–10 per observation. Values are means ± SE.
* p < 0.05 vs. baseline.
** p < 0.05 vs. 1 M HF.

that dogs paced into chronic HF (4 M HF) would demonstrate greater structural and electrophysiological remodeling than dogs paced into acute HF (1 M HF), providing a substrate for ventricular arrhythmias. We used serial echocardiography and electrocardiograms to assess ventricular function and electrical activity, respectively. Patch clamp recordings were used to measure action potentials and K^+ currents in ventricular myocytes. Real-time polymerase chain reaction (RT-PCR) was used to quantify ion channel subunit mRNA.

Materials and methods

Heart failure canine model

All animal procedures were approved by the Institutional Animal Care and Use Committee of the Ohio State University. A total of 63 adult mixed breed dogs of either sex (2– 5 years of age) weighing between 8 and 20 kg with normal cardiac function were used. Dogs were verified to have normal cardiac function by routine electrocardiograms and echocardiographic examinations during butorphanol tartrate (0.5 mg kg^{-1} intramuscularly) sedation. Dogs had a RV pacemaker lead implanted in the RV apex, and HF was induced (n = 16) by tachypacing for four months as previously described [11]. To assess time dependence during the progression of HF, echocardiograms and electrocardiogram were measured at baseline, after 1 month, and 4 months of pacing in the 4 M HF group. A second group of dogs was RV tachypaced for 1 month at 180 bpm (n = 17) and echocardiograms were measured at baseline and at the end of the pacing protocol as previously reported. [10,12]. An age matched group of 30 healthy dogs was used as controls and studied in parallel. Transmural samples of left ventricular tissue were formalin fixed and embedded in paraffin and sectioned to 5 μm thickness, using standard procedures. Tissue sections were stained with Masson's Trichrome to define the percentage area of fibrosis, as previously described [12]

Myocyte isolation

On the day of the terminal procedure, the dogs were anesthetized with pentobarbital sodium (50 mg/kg IV). The heart was rapidly removed and perfused with cold cardioplegia solution containing the following in mM: NaCl 110, $CaCl_2$ 1.2, KCl 16, $MgCl_2$ 16 and $NaHCO_3$ 10. Cannulation of the left circumflex artery was used to perfuse the left ventricle, as previously described.[11,13] Adjacent tissue samples were collected and snap frozen for protein analyses. Tyrode's solution (mM) containing NaCl 130, KCl 5.4, $MgCl_2$ 3.5, NaH_2PO_4 0.5, glucose 10, HEPES 5 and taurine 20, was used as the initial perfusate. During the cell isolation process the heart was perfused with three different solutions (36 °C). First the heart was perfused for 10 min with Tyrode's solution with 0.1 mM EGTA; followed by perfusion with Tyrode's solution containing 0.3 mM calcium, 0.12 mg/ml of trypsin inhibitor (NIBCO) and 1.33 mg/ml of collagenase (Type II, Worthington), for a maximum of 45 min. Following enzymatic digestion, the heart was perfused with normal Tyrode's solution for 5 min to remove residual enzyme. After digestion, the cells were re-suspended in incubation

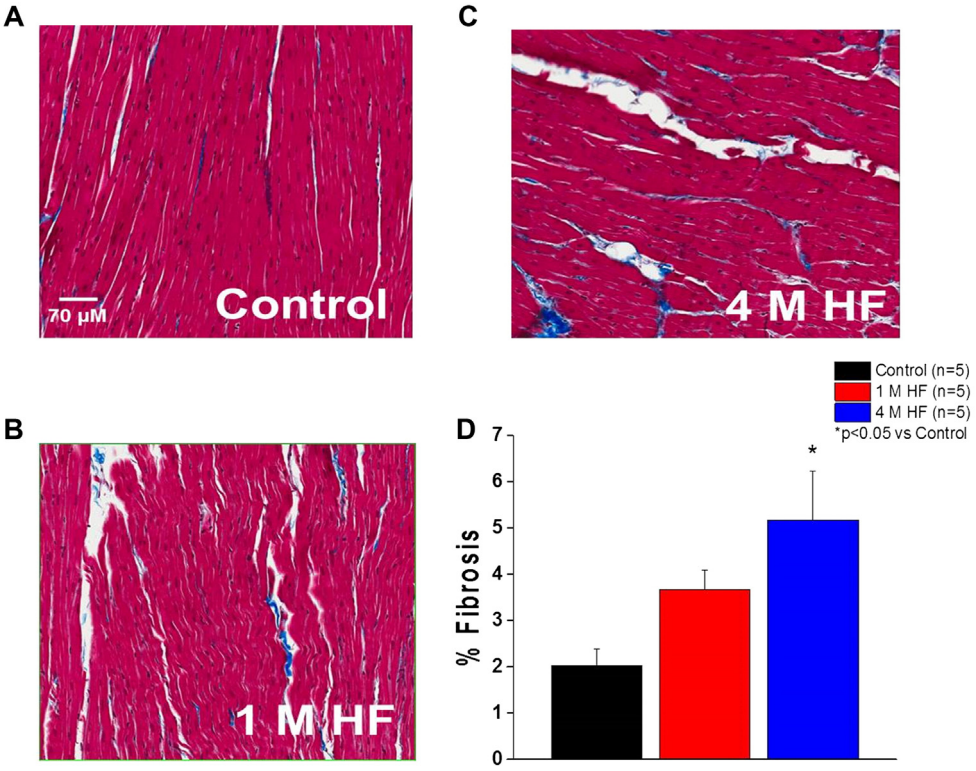

Fig. 1. Interstitial fibrosis is increased in chronic HF. Representative Masson's Trichrome staining of LV tissue. A. Control. B. 1 M HF. C. 4 M HF. D. Summary data (*p < 0.05 vs. control).