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Ventricular Effective Refractoriness Period and Ventricular Repolarization Analysis in Experimental Tachycardiomyopathy in Swine*

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Abstract

Background. Swine are recognized animal models of human cardiovascular diseases. However, little is known on the CHF-associated changes in the electrophysiological ventricular parameters of humans and animals.

Objectives. The aim of this study was to analyze changes in the durations of ventricular effective refractoriness period (VERP), QT and QTc intervals of pigs with chronic tachycardia-induced tachycardiomyopathy (TIC).

Material and Methods. The study was comprised of 28 adult pigs (8 females and 20 males) of the Polish Large White breed. A one-chamber pacemaker was implanted in each of the 28 pigs. Electrocardiographic, echocardiographic and electrophysiological studies were carried out prior to the pacemaker implantation and at subsequent 4-week intervals. All electrocardiographic, echocardiographic and short electrophysiological study measurements in all swine were done under general anesthesia (propofol) after premedication with midazolam, medetomidine, and ketamine.

Results. No significant changes in the duration of QT interval and corrected QT interval (QTc) were observed during consecutive weeks of the experiment. The duration of the QTc interval of female pigs was shown to be significantly longer than that of the males throughout the whole study period. Beginning from the 12th week of rapid ventricular pacing, a significant increase in duration of VERP was observed in both male and female pigs. Males and females did not differ significantly in terms of VERP duration determined throughout the whole study period. Ventricular pacing, stimulation with 2 and 3 premature impulses at progressively shorter coupling intervals and an imposed rhythm of 130 bpm or 150 bpm induced transient ventricular tachycardia in one female pig and four male pigs. One episode of permanent ventricular tachycardia was observed.

Conclusions. The number of induced arrhythmias increased proportionally to the severity of heart failure and duration of the experiment. However, relatively aggressive protocols of stimulation were required in order to induce arrhythmia in the studied pigs (*Adv Clin Exp Med* 2016, 25, 3, 409–414).

Key words: swine, pacemaker, tachycardiomyopathy, repolarization, refractory period.

Chronic heart failure (CHF) is one of the most common diseases of the 21st century. CHF is associated with poor prognosis; many patients with this

condition experience ventricular arrhythmias, often resulting in sudden cardiac death (SCD) [1, 2]. These clinical observations were also confirmed

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in cell culture studies and small-animal models. CHF was shown to be associated with electrophysiological remodeling, manifesting by changes in the expression of ion channels and gap-junction proteins [3]. This leads to impulse generation disorders and conduction thereof, and constitutes one of the causes of atrial and ventricular tachyarrhythmias. Also, repolarization disorders were reported in both humans and animals with CHF. The disturbances in the temporal and spatial patterns of repolarization constitute a major underlying mechanism of heart failure-associated ventricular arrhythmia [3]. However, little is known on the CHF-associated changes in the electrophysiological ventricular parameters in humans and animals. These phenomena can be analyzed using an animal model of CHF, such as experimentally-induced porcine tachycardiomyopathy.

The aim of this study was to analyze changes in the durations of ventricular effective refraction period (VERP), QT and QTc intervals in pigs with chronic tachycardia-induced cardiomyopathy (TIC). Moreover, various protocols of ventricular stimulation were compared for their potential to induce ventricular arrhythmia in the studied swine. Finally, short-term heart rate variability (HRV) and heart rate turbulence (HRT) were analyzed.

Material and Methods

The study included 28 adult pigs (8 females and 20 males) of Polish Large White breed. All animals received human care in compliance with the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health (NIH publication No. 85-23, revised in 1985 <http://www.ncbi.nlm.nih.gov/books/NBK54050/>). All experiments were performed in compliance with the Bioethics Committee of Wrocław University of Environmental and Life Sciences and the guidelines for experimentation on animals: No. 3/2008 and 40/2011.

The animals were qualified on the basis of their morphological and biochemical parameters of the blood: activity of alanine aminotransferase (AST) and aspartate aminotransferase (ALT), and urea, creatinine and ion (Na^+ , K^+ , total Ca, Mg^{2+} , Cl^-) levels. Complete blood counts were determined using a Micros ABX Vet Animal Blood Counter (ABX Diagnostics, USA) and the biochemical tests were conducted with a Konelab Prime 30i analyzer (Thermo Scientific, USA). All procedures and measurements were performed under anesthesia administered according to the same protocol, with food restriction for 12 h and water restriction for 4 h before. The pigs were premedicated with an in-

tramuscular injection of midazolam (0.1 mg/kg, Midanium 5 mg/mL, WZF Polfa S.A., Poland), medetomidine (0.02 mg/kg, Cepetor 1 mg/mL, CP-Pharma Handelsgesellschaft, Germany) and ketamine (8 mg/kg, Bioketan 100 mg/mL, Vetoquinol Biowet sp. z o.o., Poland) mixture. Anesthesia was induced by an intravenous bolus injection of propofol (2 mg/kg, Propofol 1% MTC/LCT, Fresenius Kabi, Germany). The pigs were intubated (8.5 Charriere tubes) and shaved for the surgical procedure of pacemaker implantation. Monitoring was based on noninvasive measurements of arterial blood pressure every 2 min (at the femoral artery), heart rate and pulse oximetry (tongue). A one-chamber pacemaker was implanted in each of the 28 pigs. In each animal selected for pacemaker implantation, a bipolar screw-in pacing lead was inserted into the left internal jugular vein and positioned in the myocardium at the right ventricular apex. Leads were attached to the pacemaker (Sensia, Medtronic, Ireland), and the pacing system was placed in a subcutaneous pocket. Every pig was administered antibiotic intramuscularly for infection prophylaxis for 10 days. After 2 weeks of recovery, the pacemakers were programmed for sequential right ventricular pacing at 170 beats per min (bpm), in order to develop tachycardia-induced cardiomyopathy (TIC) according to the protocol proposed previously by Paślawska et al. Electrocardiographic, echocardiographic and electrophysiological studies were carried out prior to pacemaker implantation and at subsequent 4-week intervals. Electrocardiography was performed with a BTL 08 SD electrocardiograph, with special attention paid to the parameters of repolarization (QT, QTc). Animals with electrocardiographic evidence of arrhythmia were excluded from further testing.

The transthoracic echocardiography was performed using an ALOKA 4000+ system with 5 MHz and 7.5 MHz sector probes. The following parameters were determined: left ventricular diameters in diastole (LVIDd) and systole (LVIDs), interventricular septal thickness in diastole (IVSd) and systole (IVSs), left ventricular posterior wall thickness in diastole (LVPWd) and systole (LVPWs). Ejection fraction (EF) and shortening fraction (SF) were calculated automatically. The electrophysiological study (EPS) was carried out from an external program immediately after implantation of the pacemaker. The EPS protocol included stimulation with a single premature impulse (8 + 1) in an own sinus rhythm, pacing with single, double (8 + 2), and triple (8 + 3) premature impulses at 130 bpm and 150 bpm pacing, and 60-sec stimulation at 180 bpm and 200 bpm. The aim of the pacing was to assess the time of sinus rhythm recovery (SNRT) and to provoke arrhythmia.

Statistical Analysis

The values are presented as mean $\pm$ standard deviation (SD). Differences between means were assessed by Kruskal-Wallis rank ANOVA test. All analyses were performed using STATISTICA 10.0 software. Statistical significance was set at $p < 0.05$.

Results

All of the pigs developed TIC, as confirmed by their diminished eagerness to move, gradual decrease in physical capacity, earlier appearance of dyspnea and echocardiographic abnormalities observed 4, 8, 12, 16 and 20 weeks after pacemaker implantation. Moreover, cardiac enlargement and a decrease in the shortening and ejection fractions were observed on echocardiography (Table 1). No significant changes in the complete blood count and biochemical parameters of the blood were documented during the study period.

No significant changes in the duration of QT interval and corrected QT interval (QTc) were observed during consecutive weeks of the experiment. The duration of the QTc interval of female pigs was

shown to be significantly longer than that of the males throughout the whole study period (Table 2).

Beginning from the 12th week of rapid ventricular pacing, a significant increase in duration of VERP was observed in both male and female pigs. Males and females did not differ significantly in terms of VERP duration determined throughout the whole study period (Table 3).

Ventricular stimulation with 1, 2 or 3 premature impulses at progressively shorter coupling intervals and their own rhythm did not induce arrhythmia in any of the animals, similar to the pacing with a single premature impulse at progressively shorter coupling intervals and a pacing rhythm of 130 bpm or 150 bpm. Twelve weeks after the initiation of ventricular pacing, stimulation with 2 and 3 premature impulses at progressively shorter coupling intervals and a pacing ventricular rhythm of 130 bpm or 150 bpm induced transient ventricular tachycardia in one female pig and four male pigs. Moreover, an episode of permanent ventricular tachycardia, corrected with electrical cardioversion, was observed in a male pig in the 20th week of the experiment (Table 4). None of the animals developed arrhythmia after 1-min rapid ventricular pacing at 200 bpm.

Table 1. Echocardiographic parameters determined at 4-week intervals after pacemaker implantation

Parameter	4 weeks n = 28	8 weeks n = 24	12 weeks n = 14	16 weeks n = 8	20 weeks n = 4
Ejection fraction (%)	67 $\pm$ 3.56	42.5 $\pm$ 7.45	36 $\pm$ 20.1	28.9 $\pm$ 11.1	11.4 $\pm$ 9.2
Shortening fraction (%)	37 $\pm$ 4.4	24.6 $\pm$ 7.4	20.3 $\pm$ 10.6	15.23 $\pm$ 9.6	6.23 $\pm$ 3.4
LA/Ao	1.56 $\pm$ 0.25	2.1 $\pm$ 0.2	2.42 $\pm$ 0.25	2.8 $\pm$ 0.56	3.1 $\pm$ 0.61
LVIDd (cm)	5.67 $\pm$ 0.45	6.57 $\pm$ 0.39	7.01 $\pm$ 0.9	7.34 $\pm$ 0.81	7.68 $\pm$ 0.53
LVIDs (cm)	3.55 $\pm$ 0.21	4.67 $\pm$ 0.6	5.01 $\pm$ 1.1	6.4 $\pm$ 0.9	6.62 $\pm$ 0.72

LVIDd – left ventricular internal end-diastolic diameter; LVIDs – left ventricular internal systolic diameter; LA – left atrium, Ao – aorta diameter.

Table 2. Electrocardiographic parameters of repolarization determined prior to pacemaker implantation and at subsequent 4-week intervals

Parameter	Prior to implantation n = 28	4 weeks n = 28	8 weeks n = 24	12 weeks n = 14	16 weeks n = 8	20 weeks n = 4
QT (ms) ♂	328.9 $\pm$ 33.3	328.9 $\pm$ 33.3	305.9 $\pm$ 34.2	335.8 $\pm$ 60.8	311.8 $\pm$ 43.6	317.0 $\pm$ 32.75
QTc (ms) ♂	383.7 $\pm$ 25.8*	382.7 $\pm$ 25.8*	361.5 $\pm$ 31.2*	396.1 $\pm$ 40.1*	355.9 $\pm$ 32.2*	378.2 $\pm$ 39.4*
HR ♂	83.4 $\pm$ 16.3	83.5 $\pm$ 16.3	85.3 $\pm$ 16.2	88.3 $\pm$ 35.4	79.8 $\pm$ 14.8	86.2 $\pm$ 21.3
QT (ms) ♀	323.4 $\pm$ 45.5	311.7 $\pm$ 18.5	320.2 $\pm$ 58.5	321.5 $\pm$ 17.5	352.2 $\pm$ 14	380.0 $\pm$ 23.6
QTc (ms) ♀	409.3 $\pm$ 36.4*	402.0 $\pm$ 11.1*	405.6 $\pm$ 60.0*	413.5 $\pm$ 13.9*	404.6 $\pm$ 19.8*	421.3 $\pm$ 34.6*
HR ♀	98.4 $\pm$ 18.8	100.3 $\pm$ 9.7	96.8 $\pm$ 12.5	100.2 $\pm$ 17.1	79.3 $\pm$ 16.2	75.6 $\pm$ 9.2

* statistically significant difference $p < 0.05$; HR – heart rate.

Table 3. Electrophysiological parameters determined immediately after pacemaker implantation and at subsequent 4-week intervals

Parameter [ms]	After implantation n = 28	4 weeks n = 28	8 weeks n = 24	12 weeks n = 14	16 weeks n = 8	20 weeks n = 4
♂ VERP 130 bpm 8 + 1 cycle	247.06 ± ± 39.97*	249.14 ± ± 60.08	260.85 ± ± 50.98	265.9 ± ± 31.56*	270.5 ± ± 15.6*	279.56 ± ± 14.89*
♂ VERP 150 bpm 8 + 1 cycle	233.53 ± ± 30.14*	231.03 ± ± 48.78	238.15 ± ± 55.72	242.39 ± ± 24.39	246.56 ± ± 14.8*	257.47 ± ± 11.34*
♀ VERP 130 bpm 8 + 1 cycle	245.71 ± ± 56.2*	255.54 ± ± 54.81	263.23 ± ± 24.34	272.21 ± ± 25.6*	273.43 ± ± 21.3*	276.7 ± ± 13.5*
♀ VERP 150 bpm 8 + 1 cycle	232.85 ± ± 48.2*	256.06 ± ± 44.21	253.42 ± ± 40.45	262.43 ± ± 21.4*	267.8 ± ± 19.9*	267.7 ± ± 16.7*

* statistically significant difference $p < 0.05$.**Table 4.** Echocardiographic and electrocardiographic parameters in animal with arrhythmias

Parameter	After implantation n = 5	4 weeks n = 5	8 weeks n = 5	12 weeks n = 5	16 weeks n = 4	20 weeks n = 2
Ejection fraction (%)	68 ± 4.11	66 ± 2.78	40.5 ± 9.15	38 ± 18.9	23 ± 15.6	9 ± 12.2
Shortening fraction (%)	39 ± 3.9	38 ± 5.1	24.6 ± 7.4	28.9 ± 12.4	14.6 ± 7.4	5.1 ± 4.4
LA/Ao	1.53 ± 0.2	1.54 ± 0.2	2.0 ± 0.2	2.46 ± 0.2	2.9 ± 0.6	3.2 ± 0.6
LVIDd (cm)	5.58 ± 0.42	5.63 ± 0.4	6.7 ± 0.5	7.2 ± 1.1	7.4 ± 0.78	7.74 ± 0.7
QT (ms)	321 ± 12.22	293.6 ± 41.01	328 ± 10.5	339 ± 45.46	348 ± 23.95	367 ± 29
QTc (ms)	398 ± 20.71	368.2 ± 36.44	394.4 ± 11	389 ± 34.92	403 ± 29.17	428 ± 0
HR bpm	86 ± 9.2	88 ± 13.6	87 ± 15.2	86 ± 11.6	78 ± 8.2	82 ± 6.5
VERP 130 bpm 8 + 1 cycle (ms)	no data	254.14 ± 58.8	262.35 ± 43.57	263.2 ± 36.22	272.1 ± 13.1	290.0 ± 20
VERP 150 bpm 8 + 1 cycle (ms)	no data	255.82 ± 52.5	254.5 ± 35.56	246.39 ± 34.21	251.56 ± 18.8	280 ± 20
Arrhythmias		no arrhythmias	no arrhythmias	nsVT 8 + 2 (1) nsVT 8 + 3 (4)	nsVT 8 + 2 (2) nsVT 8 + 3 (3)	sVT 8 + 2 (1) nsVT 8 + 3 (1)

Discussion

Our study differed tremendously from previously published experiments in terms of its duration (up to 20 weeks), which was sufficient to develop chronic heart failure on the basis of tachycardiomyopathy in the examined pigs. The presence of heart failure was confirmed on follow-up examinations, on the basis of significant left ventricular and left atrial enlargement, and decrease in the shortening and ejection fractions (Table 1) [4]. No significant changes in the heart rate, QT and QTc interval duration were documented during the

20-week follow-up period. However, we showed that the duration of QTc interval was significantly longer in females than in males (Table 2). This observation has also been reported in humans, and female gender is an established factor influencing the duration of the ventricular repolarization period. The duration of the QTc interval of adult men is shorter than in adult women [5]. This phenomenon is explained by the influence of sex hormones, as no significant differences in the duration of the QTc intervals of boys and girls have been observed at birth or before puberty. However, a decrease in the duration of QTc interval, by 20 ms on aver-

age, was documented in pubescent boys, whereas the duration of QTc in pubescent girls remained stable [6, 7].

We observed a progressive increase in the duration of VERP, starting shortly after the initiation of ventricular pacing, and reaching the threshold of statistical significance after 12 weeks of stimulation at 170 bpm. The duration of VERP increased throughout the whole study period, eventually being approximately 12–15% longer than at the baseline. Under physiological conditions, the duration of the repolarization period is inversely correlated with heart rate. The opposite relationship documented in our study likely reflected pathological changes associated with tachycardiomyopathy. Similar phenomenon was previously observed in tachycardiomyopathic dogs. However, in a study, conducted by Zhou et al., VERP was determined once, after 4–5 weeks of rapid pacing at 240 bpm [8]. Prolonged VERP and incomplete repolarization may contribute to the development of early after-depolarizations and trigger arrhythmias [9]. Furthermore, this may result in re-entry circuits. Heart failure is associated with prolonged ventricular action potential (APD), resulting from functional down-regulation of outward potassium currents, functional up-regulation of inward calcium current and changes in Ca²⁺ current inactivation. The prolongation of APD observed in heart failure patients is usually heterogeneous [3]. This was confirmed in a canine model of heart failure induced by rapid ventricular pacing, in which the APD prolongation of mid-myocardial cells turned out to be significantly greater than that of the epicardial cells. The heterogeneity of APD prolongation results in an increase in the transmural dispersion of repolarization [1]. The changes in transmural APD, and resultant heterogeneous and selective prolongation of repolarization, underlie an electrophysiological mechanism for unidirectional block, re-entry and arrhythmogenicity [3].

We did not find functional changes of the sinoatrial nodes of pigs with tachycardiomyopathy. Heart rate, registered at 4-week intervals, 20 min after stopping the ventricular pacing, remained within the same range throughout the whole study period. We did not observe an increase in the heart

rate that could be attributed to sympathetic activation and/or reduction of the parasympathetic tone. However, previous studies documented quantitative and qualitative changes in the parasympathetic activity, namely diminished expression of choline acetyltransferase and acetylcholinesterase mRNA, and a decrease in the acetylcholinesterase activity in the medulla oblongata [10]. The lack of functional alterations in the sinoatrial nodes might be associated with the fact that no significant changes in cardiac output were noted throughout the study period. An impairment of the left ventricular systolic function is counterbalanced by a higher pacing rate, and as a result cardiac output remains unchanged.

In our study, the number of induced arrhythmias increased proportionally to the severity of heart failure and duration of the experiment. However, relatively aggressive protocols of stimulation were required in order to induce arrhythmia in the studied pigs. We did not observe spontaneous arrhythmias during the electrophysiological examination or resting electrocardiographic recording. This is quite surprising, as the results of many previous studies of animals with experimentally-induced heart failure, including tachycardiomyopathy, and observations of human heart failure patients suggest that this condition is associated with an increased risk of spontaneous atrial and ventricular arrhythmias [2, 3, 9, 11]. There are at least two potential explanations for our findings. First, the duration of our experiment, albeit relatively long, was too short to induce profound remodeling with development of myocardial necrosis and fibrosis. The vulnerability of the muscle to spontaneously generate serious arrhythmia and the tendency to perpetuate them are associated with the presence of slow-conduction areas and non-conductive tissue zones that enable the re-entrant circuits to remain stable. This was not the case in our study group. Second, our investigation was designed to develop long-term stable symptomatic heart failure, and according to literature, these are rather short-term experiments which result in profound impairment of cardiomyocyte function. The disturbed ion channel function of such energetically compromised muscle is sufficient to develop arrhythmia [5–7].

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