“Pacing Bigeminal”

An Alternative Method in Developing a Rapid Pacing-Induced Heart Failure Model

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SUMMARY

A rapid pacing-induced heart failure model is commonly used in developing dilated cardiomyopathy (DCM). Traditionally, the right ventricular lead was connected with a single chamber pacemaker specific for animals that had a high frequency. However, the pacemaker used in this model is commercially unavailable. We developed a “pacing bigeminal” method using a commercially available dual-chamber (DDD) pacemaker to achieve high-frequency pacing. Twenty beagles were assigned to group A ($n = 10$) (pacing bigeminal method) and group B ($n = 10$) (traditional method). Echocardiographic measurements and electrocardiograms were obtained at baseline, at two weeks of pacing, and at 4 weeks of end pacing. LV anterior wall cardiac samples were obtained at 2 weeks of pacing and 4 weeks of end pacing for myocardial microscopic evaluation. Clinical manifestation and exposure time were also observed. After pacing for 10.5 ± 2.3 (7–14) days, the beagles in group B experienced heart failure, whereas in group A, only 7.9 ± 2.5 (5–12) days ($P < 0.05$) were needed to reach heart failure. Both methods could induce wide QRS duration, heart rate elevation, and myocardial microscopic changes ($P > 0.05$). In conclusion, this pacing bigeminal-induced heart failure method is feasible and can induce heart failure faster than the traditional method, which makes it a promising alternative method. (Int Heart J 2016; 57: 747-752)

Key words: Animal model, Cardiac resynchronization therapy, Dilated cardiomyopathy

Dilated cardiomyopathy (DCM) is an important cause of heart failure that is characterized by unexplained left ventricular (LV) dilatation and impaired systolic function.¹⁾ Cardiac resynchronization therapy (CRT) is a device therapy for end-stage HF, especially that caused by idiopathic DCM. In the wide application of CRT, the establishment of a suitable big-animal heart failure model is essential in basic research on DCM and CRT, because the devices are not easily used in small-animal models.

Current DCM heart failure models of large animals include myocardial infarction, coronary microembolization, pacing-induced tachycardia, and toxic injury,^{2,3)} described in detail in the AHA Scientific Statement.⁴⁾ Regional myocardial infarction models mimic human ischemic cardiomyopathy, which mainly uses pigs instead of dogs because dogs have a well-developed collateral circulation,⁵⁾ which can result in higher variability in infarct size and subsequent remodeling. Coronary microembolization also resembles human ischemic cardiomyopathy, but it is time consuming and requires repeated injection

with polystyrene microspheres. The limitations of the toxic model include variability of the response to doxorubicin and the degree of LV dysfunction and animal mortality caused by arrhythmias.⁴⁾

Chronic tachycardia-mediated DCM is a recognized clinical condition.^{6,7)} This disease model closely replicates the mechanical, structural, neurohormonal, and myocyte functional alterations of DCM in humans.⁸⁻¹¹⁾ The predictability and reproducibility of the model, and its parallels to the hemodynamic and mechanical phenotype of DCM in humans, render this an attractive model. Canines were selected in the model because of their similarity to the human cardiac structure and physiology,^{2,3)} their similar cardiac His-Purkinje system,¹²⁾ and their extensive description in tachycardia-induced CM models.⁷⁾ In the tachycardia mediated DCM model, the pacemaker is a VOO pacemaker specific for animals with high output frequency (Medtronic 8626), however, the pacemaker has become commercially unavailable in recent years.

We here developed a pacing bigeminal method using a

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two-RV lead system connected to an ordinary DDD pacemaker to excite the ventricle twice in one pacemaker beat to achieve high frequency pacing. The feasibility of the novel method was also compared with the traditional method.

METHODS

Animals and groups: Twenty beagles aged 1.4 ± 0.5 years and weighing 14.8 ± 3.2 kg (Shanghai Jiagan Biological Technology Company, Shanghai, China; License No. SCXK-Shanghai 2010-0028) were assigned to two groups. In group B ($n = 10$, mean weight, 15.9 ± 2.59 kg), one atrium lead and one ventricle lead were implanted, and the ventricle lead was connected to a high-frequency, single-chamber pacemaker used only in animals (Medtronic 8626). In group A ($n = 10$, 15.5 ± 2.22 kg), one atrium lead and two ventricle leads were implanted, and the two ventricle leads were connected to the atrial pore and ventricular pore of a DDD pacemaker (St. Jude Accent 2112), respectively. Experiments were performed using both male and female beagles at random. All animal experiments were approved by the Animal Care and Use Committee of Fu-

dan University (Shanghai, China) in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Academy Press (Eighth Edition (2011)).

Surgical procedures: The beagles were fasted for 6 hours and sedated with 3% pentobarbital sodium at 30 mg/kg; if necessary, intravenously injected succinylcholine at 10 mg/kg was used to relax muscle. All animals were intubated and mechanically ventilated with oxygen and underwent electrocardio-monitoring prior to and during surgery. To better simulate the DCM model with complete left bundle branch block, all dogs underwent left bundle branch ablation and implantation of a pacemaker in the supraclavicular area.

Group B: Under the guidance of the X line, a right ventricular (RV) lead was sutured in the RV apex, and a J type lead was sutured in the right atrial (RA) appendage (Figure 1). Intracardiac electrograms were performed to ensure the electrodes were in the endocardium of the RA and RV. The lead position was acceptable for R-wave sensing above 10 mV and pacing threshold < 2 V at 0.5 ms. The RV lead was connected with a single chamber pacemaker ventricular port (Medtronic 8626), without pacing.

Group A: under the guidance of the X line, two endocar-

Figure 1. Fluoroscopic images and ECGs. **A:** Fluoroscopic image showing the pacing electrodes of group A; **B:** Fluoroscopic image showing the pacing electrodes of group B; **C:** Fluoroscopic image showing the head placement at the beginning of the left bundle branch (LBB) in the ventricular septum; **D:** ECG after ablation showed LBBB with wide QRS duration.

dial RV leads were placed in the RV apex, and a J type lead was placed in the RA appendage (Figure 1). If the parameters were good, the two RV leads were connected to a DDD pacemaker (St. Jude Accent 2112), one inserted into the atrial port and the other into the ventricular port. The atrial lead was not connected to any port. The other procedures were the same as for group B.

Pacemaker program: The animals were allowed a 1-week period to recover from the effects of surgery, after which pacing was started. The electrocardiogram was taken after the program.

Group A: Open St. Jude programmer and enter the program interface. Set the parameters of Brady: First Step: set pacing mode to DDD, base rate to 130 bpm, A/V output to 2.5 V/0.4 ms, ventricular pacing to RV Only. Second Step: set paced atrioventricular (AV) delays as long as possible, 160 ms. Last Step: change pacing mode to DOO. The pacing VV interval is unequal, just as bigeminal rhythm (Figure 2).

Group B: Open Medtronic programmer and enter the program interface. Set the Parameters of Brady: First Step: set pacing mode to VVI, base rate to 60 bpm, A/V output to 5.0 V/0.4 ms. Second Step: go to the temporary interface and set pacing mode to VOO with the pacing rate setting at 260 bpm (Figure 2).

Observation indicators:

Baseline characteristics The operation success rate and exposure time were observed. The respiratory, heart rate, activity, and urine volume were observed, and symptoms of lethargy, decreased activity, fluid retention, or tachypnea were defined as clinical heart failure.

Electrocardiogram Electrocardiogram was performed at baseline, rapid pacing programmed, 2 weeks of pacing and 4 weeks of end pacing, the change of QRS duration and heart rate were compared.

Two-dimensional echocardiography Echocardiography was performed at baseline, after pacing for 2 weeks and after end pacing for 4 weeks (6 weeks from baseline) using a commer-

cially available system (GE VINGMED ULTRASOUND Vivid E9). The left ventricular ejection fraction (LVEF), LV end-systolic diameter (LVESd), LV end-diastolic diameter (LVESd), LV end-diastolic volume (LVESV), LV end-systolic volume (LVESV), interventricular septum diameter (IVSd), pulmonary artery systolic pressure (PASP), and the severity of mitral regurgitation (MR) and tricuspid regurgitation (TR) were evaluated using the standard criteria of the American Society of Echocardiography.¹³⁾ All echocardiographic measurements were performed during normal sinus rhythm. A single reader performed all interpretations of the echocardiographic findings and was blinded to the pacing assignment of the animals.

Myocardial microscopic evaluation LV anterior wall cardiac samples were obtained at 2 weeks of pacing and 4 weeks of end pacing. Some were stained with hematoxylin and eosin to assess myofilament, and others were fixed with glutaraldehyde for electron microscope inspection.

Statistical analysis: All data are expressed as the mean $\pm$ SD. Statistical analysis was performed using the SPSS 19.0 software package (SPSS, Inc., Chicago, IL). Comparison between groups was performed with the *t* or *t'* test for measurement data. Comparisons among the 3 time points in each group were analyzed by one-way ANOVA (Analysis of Variance). A probability value < 0.05 was considered significant.

RESULTS

Success rate of operation, complications and exposure time during operation: Twenty beagles underwent device implantation without surgical complications. Canines were randomized to group A ($n = 10$) or group B ($n = 10$). Two beagles in group A and one beagle in group B died of severe heart failure. The operation success rate was 85.7% (18/21). The exposure time for implanting the pacemaker electrode of group A was longer than that of group B (129.6 ± 20.1 s versus 84.3 ± 11.8 s, $P <$

Figure 2. Pacemaker program and ECG after programming. **A:** DOO model with paced AV delay = 160 ms was programmed; **B:** VOO model and a temporary 260 bpm were programmed; **C:** two-lead ECG showing an irregular heart rhythm with an average of 260 bpm; **D:** two-lead ECG showing a regular heart rhythm of 260 bpm.

Table I. Number of HF Beagles With Different Symptoms in Both Groups

	Decreased activity	Lethargy	Tachypnea	Hydrothorax and ascites
Group A (<i>n</i> = 8)	5	2	1	3
Group B (<i>n</i> = 9)	5	1	2	2

Table II. Comparison of the Echo Parameters at Baseline (0 weeks), 2 Weeks, and 6 Weeks in Beagles in Group A and Group B ($\bar{x} \pm s$)

	Group A (<i>n</i> = 8)			Group B (<i>n</i> = 9)		
	Baseline	2W	6W	Baseline	2W	6W
IVSd (mm)	6.9 ± 0.1	6.8 ± 0.1	6.8 ± 0.2	6.9 ± 0.1	6.9 ± 0.1	6.8 ± 0.1
LVIDd (mm)	35.22 ± 4.27	40.33 ± 4.03 [*]	39.22 ± 2.95 [#]	32.33 ± 4.27	41.00 ± 7.57 [*]	37.33 ± 6.46
LVIDs (mm)	22.67 ± 2.78	34.22 ± 4.24 [*]	29.11 ± 2.85 ^{#&}	20.67 ± 2.92	33.67 ± 6.91 [*]	27.89 ± 5.06 ^{#&}
LVEDV (mL)	33.67 ± 16.41	46.11 ± 8.45	39.89 ± 9.13 ^{#&}	32.78 ± 17.25	44.78 ± 4.60 [*]	36.00 ± 9.39 ^{#&}
LVESV (mL)	16.11 ± 7.62	33.33 ± 7.18 [*]	25.56 ± 7.80 ^{#&}	14.44 ± 8.75	31.44 ± 6.09 [*]	23.33 ± 6.50 ^{#&}
EF (%)	62.44 ± 6.06	30.56 ± 7.72 [*]	39.67 ± 3.50 ^{#&}	62.00 ± 6.36	33.44 ± 6.60 [*]	42.89 ± 5.13 ^{#&}
MR	0.33 ± 0.50	2.83 ± 0.71 [*]	1.28 ± 0.44 ^{#&}	0.44 ± 0.53	2.89 ± 0.65 [*]	1.44 ± 0.58 ^{#&}
TR	0.67 ± 0.50	3.06 ± 0.73 [*]	1.50 ± 0.56 ^{#&}	0.78 ± 0.44	2.94 ± 0.58 [*]	1.39 ± 0.49 ^{#&}
PASP	15.44 ± 12.17	34.00 ± 5.55 [*]	27.00 ± 3.77 [#]	18.67 ± 11.38	34.33 ± 6.14 [*]	27.00 ± 3.77 [#]

^{*}*P* < 0.05 versus baseline, [#]*P* < 0.05 versus baseline, [&]*P* < 0.05 versus 2W. 2W indicates pacing 2 weeks; 6W, end pacing of 4 weeks (6 weeks from baseline), Group A, “pacing bigeminal” method; Group B, the traditional method; EF, ejection fraction; MR, mitral regurgitation; TR, tricuspid regurgitation; IVSd, interventricular septum diameter; and PASP, pulmonary artery systolic pressure.

0.05). After pacing 10.5 ± 2.3 (7-14) days, the beagles in group B had signs of overt heart failure, such as lethargy, decreased activity, fluid retention, or tachypnea, whereas in group A, only 7.9 ± 2.5 (5-12) days (*P* < 0.05) were needed to produce signs of heart failure (Supplemental Figure 1, Table I).

Electrocardiogram change: The changes of heart rate and QRSd are shown in Supplemental Figure 2. There were no differences between the two groups.

Echocardiographic performance: The echocardiographic findings are summarized in Table II. Three beagles in group A and 2 beagles in group B had mild to severe mitral and tricuspid regurgitation (semiquantitative, zero to 4+) and increased pulmonary artery systolic pressure (PASP). There were no differences between the two groups.

HE staining: After pacing for 2 weeks and end pacing of 4 weeks, the LV free walls of the beagles were removed for pathological section examination by light microscopy. The results showed that the myocardial region of both groups had small vascular congestion, myofibrillar thinning, and irregularly arranged muscle fibers. After 4 weeks of recovery, the muscle fiber was thicker and was improved compared with pacing for 2 weeks, but it was still damaged compared to normal myocardium (Supplemental Figure 3). We measured myofibrillar thickness, but there was no significant difference between the two groups (Supplemental Figure 4).

Electron microscopic changes: After pacing for 2 weeks, cellular edema, a decreased number of mitochondria, mitochondrial swelling, myofibrillar thinning, and disordered intercalated discs were found in both groups. After 4 weeks of recovery, the mitochondrial edema was improved, but it was not completely restored to normal (Figure 3).

DISCUSSION

The possible mechanisms of traditional rapid pacing-induced heart failure include: 1) high heart rate. A heart rate of

twice the basal heart rate causes the myocardium to sustain rapid contraction without sufficient relaxation. Decreased cardiac output, increased LV end diastolic pressure, and shortened diastolic perfusion time lead to reduced coronary perfusion and decreased myocardial oxygen supply.¹⁴⁾ This suggests that ischemia and hypoxia injury are present in the process of rapid pacing-induced heart failure. 2) pacing-induced LV dyssynchrony. RV apex pacing changes the order of ventricular activation, from the apex to the bottom of the heart, not by the Purkinje system, which causes the electrical and mechanical activity of the atrio-ventricle and intra- and inter-ventricle. The abnormal pattern of electric activation and LV dyssynchrony may cause disruption and further progression of dyssynergic LV wall motion.¹⁵⁻¹⁷⁾

Here we introduce a novel method to develop a HF model, in which two ventricular leads were implanted in the right ventricle, connected to the pacemaker atrial and ventricular holes, excites the ventricle twice in one pacemaker beat and doubles the heart rate, using a proper coupling interval and basic heart rate, which is different from traditional rapid pacing. Extending the AV interval makes the ventricular rate as smooth as possible. However, the AV interval is not unlimited, and the rhythm is like the premature ventricular bigeminal. We called it “pacing bigeminal”. In addition to the two theories, similar to the traditional rapid pacing, another theory is that ectopic ventricular activation was caused by the limited AV delay. Ectopic ventricular activation is eccentric, leads to dyssynchronous contraction,¹⁸⁾ and induces LV dysfunction in otherwise normal hearts.¹⁹⁾ In addition, the pauses following the ectopic beats impact ventricular filling and emptying dynamics²⁰⁾ and may cause the chronic effects of “postextrasystolic potentiation”.¹⁹⁾ It is known that AF may compromise LV systolic function through poor rate control and irregularity of the ventricular response.²¹⁾ Therefore, the variance and irregularity of heart rate in our new method could be another way to explain the induction of heart failure. Given the above, we believe that the mechanism of this pacing bigeminal shares some similarities

Figure 3. Electron microscopic images showed mitochondria changing with pacing time in both groups compared with normal beagle myocardium.

ties to other clinical entities, such as tachycardia-induced cardiomyopathy, pacing-induced cardiomyopathy, and PVC-induced cardiomyopathy, and can be more rapid and stable for establishing a heart failure model.

Negative remodeling of the left ventricle of a failed heart can result in complete LBBB and a prolonged QRS duration.²²⁾ To better simulate the DCM-heart failure model, all dogs in this experiment underwent left bundle branch ablation; thus, QRS duration was longer than in normal beagles, which may influence the conduction system and further cause left ventricular asynchrony and heart failure. In addition, electron microscopic examination showed the mitochondria were swollen in both groups, which suggested that the dysfunction of mitochondrial function played an important role in the process of heart failure.²³⁾

This new method used one more lead than the traditional method, so the exposure time was longer than the traditional method. The new method also requires a specific pacemaker (so the AV interval can be fully elongated), which increased the cost. Another limitation of our study is that we did not implant two leads in the control group to make the conditions the same in the two groups. The leads implanted in the RV system are likely to induce tricuspid regurgitation and RV heart failure. However, RV heart failure has minimal impact on the LV function, which is our focus. Actually, there were no differences in the severity of tricuspid regurgitation between the two groups in our study. Other studies showed that in the short term the electrodes had almost no effect on cardiac function.^{24,25)} Therefore, we believe the difference in the number of RV leads does not affect the reliability of our results.

In conclusion, we have shown for the first time that pacing bigeminy-induced heart failure is feasible and can induce heart failure faster than the traditional method, which makes it a promising alternative method.

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Conflict of interests: All authors declare that they have no any conflict of interests.

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SUPPLEMENTAL FILE

Supplemental Figures 1, 2, 3, 4

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