國家科學委員會專題研究計劃報告

計劃名稱：

慢性心房顫動的豬模式建立及心房電氣活動分析：

心房的線形切割策略評估

Establishment and Computerized Mapping of Chronic Atrial Fibrillation in Pigs: Evaluation of Linear Ablation Strategy

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Introduction

The best cost-beneficial strategy in the management of atrial fibrillation is the restoration and maintenance of sinus rhythm. However, despite vigorous efforts in multiple therapeutic approaches, the eradication of atrial fibrillation remains difficult. The only exception is the atrial maze or compartmentation surgery, which dissects the atria into several communicating or non-communicating parts. These surgical procedures recover the sinus rhythm with adequate atrial transport in over 85% of patients with chronic atrial fibrillation.

In order to replicate the success by surgery, multiple innovated ablation devices with or without associated new energy sources are sprouting actively to overcome multiple defects of contemporary catheter ablation techniques, including the effective and efficient creation of linear lesions over the atrial wall or circumferential lesions at the venous orifices, the acute and long-term safety of complex atrial ablation lesions and the appropriate application of new ablation devices or systems etc. Meanwhile, de novo concepts in the construction of new antifibrillatory drugs as well as in the targeted delivery of antiarrhythmics based on unique electrophysiological characteristics of atrial fibrillation are also reviving in the pharmacological approach. All aforementioned new developments in ablation technologies, pharmaceutics or even new ablation protocols, nevertheless, necessitate convincing evidences about their feasibility, efficacy and possible complications before clinical practice A reproducible and reliable animal model of sustained atrial fibrillation simulating that in human diseases is therefore critical.

In the present study, we demonstrated the consistent and efficient creation of chronic sustained atrial fibrillation in a new swine model, which possesses similar

electrophysiological and histological characteristics as that in humans. We believed the highly reproducible swine model would serve as an excellent experimental model suitable for the research and verification of new therapeutic modalities in the definitive therapy of chronic atrial fibrillation in humans.

Materials and Methods

Study animals

Twenty-eight adult pigs of Yorkshire-Landrace strain were used. The mean body weight was 75 ± 14 kg (range 60 to 94 kg). The experimental protocol conformed to the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996) and approved by Institutional Animal Care and Use Committee.

Creation of swine atrial fibrillation by atrial high-speed pacemaker

The 28 pigs were separated into the study group (25 pigs) and the sham group (3 pigs). Baseline surface ECG and two dimensional transthoracic echocardiography were recorded in advance to measure cardiac performance and to exclude associated cardiac abnormality. Subsequently, under light anesthesia by intravenous thiamylal (Kyorin Pharmaceutical Co., Tochigi, Japan) or ketamine (Shin-Tong Pharmaceutical Co., Taoyuan, Taiwan), all animals were implanted with a transvenous high-speed atrial pacemaker (Itrel-II, Medtronic, Minneapolis, Minnesota, U.S.A) for the study group or an inactive pacemaker for the sham group. The atrial pacing lead with screw-in tip (Medtronic) was inserted via the internal jugular vein and fixed at the atrial appendage under fluoroscopy. Local threshold, impedance and P-wave sensing of the atrial lead were tested before connecting to the pacemaker generator which was then positioned below the chin of the

pig. Total procedure was generally completed within 20 minutes for each animal. After the closure of the pacemaker pocket, the animal was allowed to recover from anesthesia and returned to the individual dormitory. Three days later, the atrial pacemaker was activated to the programmed rate of 600 beats per minute (bpm) by a telemetry programmer (Medtronic). Oral digoxin (0.25mg per day) was given from the first day to prevent rapid heart rate and possible congestive heart failure during programmed atrial pacing. The high-speed atrial pacing was continued for a total of six weeks. Consistency of the atrial pacing was checked in the dormitory by a portable ECG monitor one to three times a week.

Electrophysiological study of swine atrial fibrillation

After continuous atrial overdrive pacing at 600 bpm for six weeks, the atrial pacemaker was shut off by the telemetry programmer. Usually, spontaneous sustained atrial fibrillation would emerge. According to the protocol, we confirmed the atrial fibrillation and let persist for 24 hours in 1/3, 48 hours in 1/3 and 72 hours in 1/3 of the survivals. The persistence of atrial fibrillation was monitored by twice daily ECG records Transthoracic echocardiography was repeated after the discontinuance of atrial pacing. Surface ECG monitoring was followed once to twice daily before the final study.

For the final study, animals of either the study group or the sham group were returned to the animal laboratory. They were intubated via tracheostomy and ventilated artificially by a modified volume respirator (tidal volume 10 ml/kg, respiration rate 20-25/min) after full sedation with intravenous thiamylal. Right thoracotomy was performed and the pericardium was cradled to expose the atrial free surface.

Epicardial mapping of the sustained atrial fibrillation was performed by a rectangular

recording plaque (Prucka Engineering Inc., Houston, Texas, U.S.A) with 224 (14 x 16) sites of copper electrodes, which produced 224-site bipolar recordings by paired connections. The intra- and inter-bipolar distances were 3.5 mm. The epicardial mapping was started from the right atrium and then the left atrium. All 224-site local atrial electrograms and the surface ECG were monitored and recorded simultaneously by a computerized multi-channel mapping system (CardioMapp™, Prucka Engineering Inc.). The data were acquired at 1000 samples per second. Each event recording was continued for 30 seconds.

Pharmacological study. Intravenous dl-sotalol (Bristol-Myers-Squibb GmbH, Munich, Germany) at dosage of 2 mg/kg was given in 6 animals to test the possibility of acute termination. If atrial fibrillation persisted, both the right and left atria were mapped again by the plaque electrode to evaluate the change of atrial activation patterns after the addition of dl-sotolol.

Interventional study by atrial compartmentations. To evaluate the stability and atrial dominance for the sustained atrial fibrillation induced in the swine model, direct compartmentation of the right atrial free wall (6 pigs) plus left atrial appendage (4 pigs) was performed by epicardial radiofrequency (Radionics, RFG-3C, Burlington, Massachusetts) and cryothermal ablation (Cryounit 142, Spembly Medical Corp., Andover, United Kingdom) in 8 animals. The application of radiofrequency energy was transmitted through the 4 mm tip electrode of standard ablation catheter and monitored by local impedance, voltage, current, power and epicardial discoloration. On the other hand, the cryoablation was applied by a T-shaped ablation pencil at -60℃ for 180 seconds for each target. For the compartmentation of right atrial wall, linear ablation lesions were

connected from the orifice of superior vena cava to that of inferior vena cava and to anterolateral part of the tricuspid annulus, including the right atrial appendage. If atrial fibrillation persisted, another ablation line was circled around the orifice of left atrial appendage to isolate it. The success of the atrial compartmentalizations was confirmed by the appearance of electrical silence or dissociation inside of the isolated atrial tissue by plaque electrode mapping.

Pathological and histological examinations. At the end of study, the animals from either the study or the sham group were euthanized by high-dose intravenous pentobarbital. The previously implanted atrial pacemaker was removed and the heart was excised and weighed immediately. Atrial tessues from multiple atrial locations were sampled for later light and electron microscopy examinations.

Data analysis.

The activation pattern of atrial fibrillation was constructed by a custom-made multichannel analysis program (EMAP, Uniservice, Auckland, New Zealand) after reconversion of the digitized data stored in the recording system (CardioMapp). The time of activation of each local electrogram was first assigned automatically at the maximum dv/dt and subsequently edited manually. The mininum of local A-A intervals was arbitrarily selected as 60 msec. The pattern of activation was then demonstrated as dynamic illuminations on a computer screen. Whenever an activation was registered, the corresponding electrode site was illuminated as initially red, then yellow, green, light blue and finally dark blue. The cinematic display help identify individual activation wavefronts and activation pattern in the recording plaque. The number of activation waves was summed and averaged over the recording time after repeated display of the activation

illuminations in 5 or 10 msec steps. In case of wavefront break-up, each daughter wavelet was counted as a new activation wave. The activation analysis of atrial fibrillation was performed for both right and left atrial plaque recording in baseline condition, after dl-sotalol use, and after interventional procedures.

Statistics.

Continued data were expressed as mean SD and compared by student's paired or unpaired *t* test.

Results

Production of sustained atrial fibrillation by programmed atrial overdrive pacing in pigs

Twenty-two (88%) of the 25 study group pigs and 3 (100%) of the 3 sham group pigs survived the process of atrial pacemaker implantation and the 6 weeks of continuous atrial overdrive pacing. Three pigs of the study group died in the post-implantation period due to pneumonia and sepsis in one pig, acute volvulus in one and intractable congestive heart failure in one. The body weight of the survived pigs of the study group increased markedly from 75±14 kg (range 52 to 90 kg) to 100±16 kg (range 80 to 125 kg), without evidence of central or peripheral fluid retention. The weight gain was similar in that of the sham group, i.e. from 70±8 kg (range 62 to 80 kg) to 95±9 kg (range 76 to 110 kg).

After the discontinuance of high-speed atrial pacing, spontaneous persistent atrial fibrillation was documented in 20 of the 22 survived pigs in the study group, but in none of the sham group. The atrial fibrillation was let persisting for 24 hours in 6 pigs, 48 hours in 6 pigs and 72 hours in 8 pigs before final study. Two remained pigs in the study group

had nonsustained atrial fibrillation for less than 24 hours.

Transthoracic echocardiography before and after the continuous atrial pacing revealed significant increase of the left atrial dimension (before vs. after, 26 ± 3 mm vs. 31 ± 4 mm, P = 0.01), but not the end-systolic (29 ± 4 mm vs. 32 ± 6 mm, P = 0.28) and the end-diastolic (49 ± 5 mm vs. 53 ±9 mm, P = 0.26) dimensions of left ventricle nor the fractional shortening (40 ± 10% vs. 39 ± 8%, P = 0.77) and the ejection fraction (69 ± 11% vs. 67 ± 10%, P = 0.60) of left ventricle.

Bi-atrial epicardial mapping of spontaneous sustained atrial fibrillation in pigs

All of the 22 survived pigs from study group had continuous atrial fibrillation, either spontaneous (20 pigs) or induced (2 pigs), throughout the open-chest epicardial mapping study. The 2 pigs with nonsustained atrial fibrillation could be easily induced of persisting atrial fibrillation by one to three extrastimuli from right (1 pig) or left atrium (1 pig).

All of the spontaneous atrial fibrillation produced in the study group pigs revealed the coexistence of multiple reentrant wavelets. The number of activation wavefronts in atrial fibrillation was more frequent over left atrial free wall (10.6 ± 2.9 wavelets/cm^2/sec) than that over right atrial free wall (7.6 ± 2.4 wavelets/cm^2/sec, P < 0.002) by dynamic display analysis of the plaque-recorded activation sequences. None of the activation wavefronts of spontaneous atrial fibrillation in the pig model could be demonstrated to complete one reentrant rotation by either left atrial or right atrial epicardial mapping. The mean local A-A intervals were 86.3 ± 14.2 msec in the left atrium and 102.1 ± 17.8 msec in the right. The coefficiency of variance was 16.4% in the left atrium and 17.4% in the right.

The spontaneous atrial fibrillation developed in the pig model was not terminated by iv. dl-sotalol in any of the 6 tested animals. The mean local A-A intervals after sotalol was

114.9 ± 24.8 msec in the right atrium and 100.7 ± 22.3 msec in the left. The number of coexisting activation wavefronts was similar over the right and the left atrial epicardial surface (6.5 ± 3.5 vs. 9.3 ± 3.4 wavelets/cm^2/sec, right vs. left atria, P=0.13).

Discussion

In the study project, we have successfully established the adult pig model of chronic atrial fibrillation. The experimental atrial fibrillation can be produced with high efficiency. Most importantly, the induced atrial fibrillation consisted of multiple, reentrant wavelets in electrophysiology, without evidence of macro reentrant circuit pathway, mimicking long-term atrial fibrillation in humans.

Currently, the interventional therapy for atrial fibrillation has been largely hampered by the ineffectiveness and inefficiency of transcatheter ablation systems in regards of transmural creation of linear or circumferential lesions in the atria. Despite the success in surgical approach, transcatheter intervention remains unable to reproduce the requested ablation design, e.g. maze procedure, atrial compartmentalization, or even limited posterior left atrial isolation etc. Our pig model apparently provided the best platform for the future in vivo tests for new technological breakthroughs. The atria of adult pigs usually possess thick pectinate muscle and atrial free wall similar to that in chronic human atrial fibrillation, with or without associated cardiovascular disease. The multiple, reentrant, but meandering activation wavelets in pig atrial fibrillation reflects the true electrophysiological situations in human counterpart and could accurately testify the success of failure of future interventional devices in the elimination of the complex atrial dysrhythmia. The most important things in the experimental point of view are of course

the high harvest rate and the short incubation period in the adult pig model for production of chronic sustained atrial fibrillation. This is definitely better than the mongrel dog model which has only 20% harvest rate after 8 weeks of continuous atrial high-speed pacing.

Basically, the success of adult pig model is the reproduction of the critical mass theory, which states that a critical mass is important for the maintenance of fibrillation in the heart. Also, the large atrial mass in adult pigs is compatible with the Moe-Allessis's hypothesis emphasizing the need of multiple wavelet and a critical wavelet number in the maintenance of sustained atrial fibrillation. However, the basic cellular or molecular mechanism underlying the propensity or early development of long-lasting atrial fibrillation can't be thoroughly documented in this experiment. Further investigations about the I_{Kr}, I_{Ks}, I_{Na}, I_{Ca} or even I_{to} current channels, channel distribution, and relevant anatomical or functional barriers are crucial for the understanding of the initiation and maintenance of long-term atrial fibrillation in this pig model. We believe such investigations will help clarification of multiple conceptual defects.

As to the strategy in ablation of atrial fibrillation, we could demonstrate several findings in our pig experiment. First, the compartmentalization of most of the right atrium couldn't stop atrial fibrillation, not even with the combination of left atrial appendage; Second, the compartmentalized part of the atrium manifested regular, flutter-like activation with slower cycle length (250-300 msec); Third, the posterior left atrium or the convergence area of the pulmonary veins may be crucial to the maintenance of atrial fibrillation; Fourth, the creation of transmural atrial lesions is difficult and most likely related to the pectinate muscle ridges, below-ridge space, or intra-atrial folds. The design of ablation lines has to take this anatomical obstacles into serious consideration. For

example, the circumferential ablation at the pulmonary vein orifices from inside of left atria may be a good idea to bypass the obstacles.

In conclusion, in the past year, we have successfully created an adult pig model of chronic sustained atrial fibrillation, which possessed important characteristics of human counterpart in terms of electrophysiology and histo-anatmical details. The model has high harvest rate and suitable anatomical environment for the experimental tests of all future interventional designs or conceptual breakthroughs.