國家科學委員會專題研究計劃報告

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運用三度空間磁場向量導引定位以電燒灼治療持續性心房顫動的可行性及病理、電生理機轉：成年豬模式

Three-Dimensional Electromagnetic Navigation Mapping and Linear Ablation of Swine Sustained Atrial Fibrillation: Feasibility, Pathology and Mechanisms of Ablation Outcome

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Introduction

It is the general consensus that restoration and maintenance of sinus rhythm is the best cost-effective strategy in the management of atrial fibrillation (AFib)[1]. Despite recent multiple innovated therapeutic approaches[2-8], long-term elimination of AFib remains difficult, especially in the setting of chronic persistent AFib. In conjunction to these therapeutic advances in humans, several in-vivo and in-vitro animal models have been used to study the mechanisms and interventional modalities of AFib[9-15]. However, an animal model with sustained AFib ([9, 15], which is closely similar to that 24hours) humans in the electrophysiological and histological properties, remains quite limited and hence would be highly desirable.

This study was undertaken to develop an animal model of sustained AFib in pigs, to assess its characteristics by performing electrophysiological mapping during AFib, histological examinations of the atrium and to perform epicardial atrial ablation therapy for the persistent AFib.

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 the Institutional Animal Care and Use Committee of the National Taiwan University College of Medicine. All pigs were provided by and housed at the animal facility in the Pig Research Institute in Taiwan (PRIT) in Chunan, Taiwan. The animal experiments were conducted at the PRIT (pacemaker insertion) and in the animal catheterization laboratory at the National Taiwan University Hospital (electrophysiological studies and mapping).

Sustained atrial fibrillation induced by rapid atrial pacing.

Of the 28 pigs, 25 were entered into the study group and 3 in the sham group to serve as a control. Baseline surface 12-lead ECG and transthoracic echocardiography were performed to evaluate cardiac function and to exclude any animals associated with cardiac abnormality. Under intravenous anesthesia by thiamylal (2-3 mg/kg) (Kyorin Pharmaceutical Co., Tochigi, Japan) or ketamine (1-2 mg/kg) (Shin-Tong Pharmaceutical Co., Taoyuan, Taiwan), each animal was transvenously implanted with either a high-speed atrial pacemaker (Itrel-II and Itrel-III, model 7424 and 7425, Medtronic, Inc., Minneapolis, Minnesota, U.S.A) for the study group or an inactive pacemaker for the sham group. The atrial pacing lead (Model 4568, Medtronic Inc.,) was inserted via the left internal jugular vein by a cut-down technique and screwed to the right atrial appendage or the right anterior atrial wall under fluoroscopy. Pacing threshold, impedance and P-wave amptitude of the atrial lead were tested before connecting to the pacing generator which was

implanted subcutaneously in the neck and below the chin of the pig. After closure of the pacemaker pocket, the animal was left recovering from anesthesia and returned to the pig housing dormitories. Three days later, the atrial high-speed pacemaker was programmed to a rate of 600 beats per minute (bpm) for a total of six weeks. Oral digoxin (0.25mg per day) was given daily from the first day after pacemaker implantation to minimize a rapid heart rate response and possible congestive heart failure. Consistency of the atrial pacing was regularly checked daily in the first week and weekly thereafter by a portable ECG monitor.

Electrophysiological mapping study.

After six weeks of continuous pacing, the atrial pacemaker was turned off by a programmer. Transthoracic echocardiography was repeated to evaluate the changes of cardiac function. Subsequently, animals underwent electrophysiologic mapping studies in a random order in day 1, day 2, or day 3 after termination of atrial pacing.

Each animal was intubated via tracheostomy and ventilated artificially by a modified Harvard respirator (tidal volume 10-15 ml/kg, respiration rate 20-25/min) after full sedation with intravenous thiamylal (initial 5 mg/kg, then 50-100 mg intermittently). Right thoracotomy was performed and the pericardium was cradled to expose the atrial free surface.

Epicardial mapping of activation sequences of AFib was performed by a rectangular

recording plaque electrode (62×52 mm^2) (Prucka Engineering Inc., Houston, Texas, U.S.A), which contains 224-site bipolar recordings by paired connections. The intra- and inter-bipolar distances were 3.5 mm. The epicardial mapping was conducted sequentially on the right and the left atrium (Figure 1). All 224-site atrial local electrograms and surface ECG were monitored and recorded simultaneously by a computerized multi-channel mapping system (CardioMapp^{TM}, Prucka Engineering Inc.). The data were acquired at 1000 samples per second. Each event was recorded continuously for 30 seconds.

Pharmacological study.

Intravenous dl-sotalol (1.5 mg/kg) (Bristol-Myers-Squibb GmbH, Munich, Germany) or propafenone (2 mg/kg) (Knoll SA, Liestal, Germany) was given in 11 animals (sotalol in 6, propafenone in 5) to assess the drug effects. Both right and left atria were re-mapped by the plaque electrode after the addition of antiarrhythmics.

Interventional study.

To evaluate the effect of a linear ablation technique, regional compartmentalization of either the right atrial free wall alone (4 pigs) or together with the left atrial appendage (4 pigs) was performed by epicardial cryothermal linear ablation (Cryo-unit 142, Spembly Medical Corp., Andover, United Kingdom). The cryoablation lesions were applied consecutively by a T-shaped ablation probe at -60℃ (for 60 sec each) along the designed

compartmentalization lines[3]. For isolation of the right atrial free wall, ablation lines were connected from the orifice of the superior vena cava to that of the inferior vena cava as well as to the anterolateral aspect of the tricuspid annulus, including the right atrial appendage. If AFib persisted, another ablation line was applied to encircle the orifice of the left atrial appendage. The effectiveness of atrial cryothermal compartmentalization was confirmed by demonstration of independent activation wavefronts in each of the compartmentalized atrial tissue by plaque electrode mapping.

Pathological and histological examinations.

At the end of study, the animals were euthanized by high-dose intravenous barbiturate. The atrial pacemaker was removed and the heart was excised and weighed and inspected immediately. All the cryoablation lines were examined grossly and histologically. Tissue blocks from multiple locations including the appendages, the lateral walls, the posterior walls and the septum of both atria were excised for light and electron microscopic examinations.

Data analysis.

The activation sequences of AFib were analyzed by a custom-made multichannel analysis program (EMAP, Uniservice, Auckland, New Zealand)[15, 16] after redigitalization of the data stored in the recording system. The activation time of each local electrogram on each recording channel was assigned automatically at the maximum dv/dt and

subsequently edited manually. The minimal local A-A intervals in the local activation time was chosen as 60 msec, representing the minimal acceptable atrial refractoriness[15]. The pattern of activation was then displayed as dynamic sequential illumination on a computer screen. Whenever an activation was registered, the corresponding electrode site illuminated as initially red, then yellow, green, light blue and finally dark blue. The repeated 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 total recording time after repetition of the dynamic 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 AFib was performed for both right and left atrial plaque recordings at baseline, after antiarrhythmic drugs, and after cryothermal interventions.

Statistics.

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

Results

Induction of sustained AFib in adult pigs

Twenty-two (88%) of the 25 pigs in the study group and 3 (100%) of the 3 pigs in the sham group survived 6 weeks after the implantation of atrial pacemaker. Three pigs in the

study group died during follow-up period due to pneumonia in one pig, acute volvulus in one and intractable congestive heart failure in one. The body weight of the survived pigs in the study group increased from 75±14 kg (range 52 to 90 kg) to 100±16 kg (range 80 to 125 kg) in 6 weeks without evidence of fluid retention. The weight gain was similar in the sham group, i.e. from 70±8 kg (range 62 to 80 kg) to 95±9 kg (range 76 to 110 kg).

After discontinuance of atrial pacing, sustained AFib (

(91%) of the 22 survived pigs in the study group, but in none of the sham group. AFib was left persistent for 24 hours in 6 pigs, 48 hours in 7 and 72 hours in 7 prior to the electrophysiological mapping. Two pigs in the study group had nonsustained AFib lasting less than 24 hours after termination of pacing.

Transthoracic echocardiography before and after continuous atrial pacing revealed significant increase in the left atrial dimension (26 ± 3 mm vs. 31 ± 4 mm, P = 0.01), but not in the left ventricular 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, or the left ventricular fractional shortening (40 ± 10% vs. 39 ± 8%, P = 0.77) and the ejection fraction (69 ± 11% vs. 67 ± 10%, P = 0.60).

Bi-atrial epicardial mapping of sustained AFib

All of the 22 survival pigs in the study group had persistant AFib throughout the open-chest epicardial mapping study. The two pigs with nonsustained AFib could be

easily induced into persistent AFib by one to three electrical extrastimuli from the right or the left atrium. The mean ventricular rate was 77 ± 3 bpm in the 20 pigs with sustained AFib, and 74 bpm in the other two pigs with paroxysmal AFib.

All of the AFib revealed the coexistence of multiple reentrant wavelets by epicardial mapping. The wavelets propagated, migrated, collided and generated new daughter wavelets. None of the wavelets circulated in a stable pathway. The number of activation wavelets recorded at the left atrial free wall (10.6 ± 2.9 wavelets/cm^2/sec) was generally higher than that at the right atrial free wall (7.6 ± 2.4 wavelets/cm^2/sec, $P < 0.002$), as calculated by dynamic analysis of activation sequences. The mean local A-A intervals were 87.2 ± 14.6 msec in the left atrium and 103.3 ± 19.0 msec in the right atrium ($P < 0.0002$). The mean coefficiency of variance was 7.4 ± 3.9% in the left atrium and 7.9 ± 4.1% in the right atrium ($P = 0.77$).

Pharmacological study

Sustained AFib induced in the present model could not be terminated by intravenous dl-sotalol in any of the 6 tested animals. The mean local A-A intervals after dl-sotalol did not change significantly either in the left atrium (102.4 ± 20.3 msec, vs. 89.4 ± 12.6 msec, $P= 0.11$) or in the right atrium (120.8 ± 29.6 msec, vs. 99.8 ± 13.1 msec before dl-sotalol, $P = 0.10$). The number of co-existing activation wavelets was similar before and after the use of sotalol (left atrium, 10.7 ± 3.8 vs. 9.3 ± 3.4 wavelets/cm^2/sec, $P= 0.21$; right atrium,

7.2 ± 2.2 vs. 6.5 ± 3.5 wavelets/cm^2/sec, P = 0.34). Activation wavefronts remained meandering and unpredictable in dynamic display.

In contrast, intravenous propafenone terminated AFib in 3 of the 5 study animals. All of the acutely terminated AFib could be immediately reinitiated by one to three electrical extrastimuli to the left atrial wall. The mean local A-A intervals after propafenone, measured during sustained AFib in two pigs or reinduced AFib in three, prolonged significantly in the right atrium (143.0±30.5 ms vs. 107.8±16.0 ms, after vs. before propafenone, P<0.05), but not in the left atrium (141.3±58.2 ms vs. 96.8±16.2 ms, P=0.14). In dynamic display, propafenone apparently reduced the number of reentrant wavelets at both the left atria (4.5±2.0 vs. 10.4±3.2 wavelets/cm^2/sec, after vs. before propafenone, P<0.05) and the right (2.1±1.2 vs. 8.0±3.1 wavelets/cm^2/sec, after vs. before propafenone, P<0.04). The activation wavelets after propafenone appeared more organized and rotated in a larger radius, although the migratory characteristic of the wavelets remained unchanged.

Atrial compartmentalization by epicardial cryoablation

Despite effective cryo-compartmentalization in the right atrial lateral wall alone (4 pigs) or together with the left atrial lateral wall (4 pigs), sustained AFib did not terminate in any of the 8 animals receiving this interventional proceduce. Direct epicardial recordings confirmed effective compartmentalization of the atrial tissue by demonstration

of independent regular activation wavefronts inside the compartments. The mean local A-A interval was 285.7 ± 30.4 msec inside the isolated right atrial free wall (8 pigs) and 260.2 ± 28.1 msec inside the isolated left atrial appendage (4 pigs). The atrial wall beyond the cryoablation lines remained in multiple reentrant wavelet activations, with mean local A-A intervals of 105.5 ± 40.5 msec in the spared left atrium after right atrial compartmentalization ($P < 0.02$, vs. the isolated right atrial wall).

Pathological and histological findings

The weight of the excised hearts was 550 ± 14 gms (range 480 to 670 gms) in the study group and 408 ± 10 gm (range 370 to 430 gm, $P<0.03$) in the sham group. Serosanguious pericardial effusion ranging 30 to 120 ml was found in 10 pigs in the study group. None were found to have atrial perforation due to a penetrating atrial lead. The cryoablation lines over the right atrial anterior and posterior walls and the root of the left atrial appendage were all transmural by gross inspection. No atrial thrombus was found.

Light microscopic examination showed no evidence of patchy or interstitial myofibrillar fibrosis in either the right or left atrium. However, under electron microscopy, the atrial myocytes from both atria in the study group revealed diffuse intracellular changes including prominent perinuclear myolysis, scattered myofilament fragmentation, mitochondrial cristae disruption, mitochondrial emptying with compensatory proliferation, which were not observed in the sham group.

Discussion

Main findings.

Our study demonstrated a reliable swine model that is easily induced into sustained AFib by rapid atrial pacing, and which has similar electrophysiological and histological characteristics to that seen in humans. This AFib could not be completely suppressed by intravenous dl-sotalol, propafenone, or by atrial compartmentalizations with our methods. The electrical and anatomical resemblance of the present swine model to that seen in humans might be useful to serve as an excellent tool for future investigations of the mechanism and therapy of sustained AFib.

Comparison with previous studies.

Until now, there is still no single therapeutic approach universally accepted for the elimination of AFib. Multiple experimental animal models have been developed for various investigational purposes[9-12, 15].

With continuous rapid atrial pacing for 6 weeks, our pigs could be easily induced to sustained AFib lasting for at least 24 hours. The swine AFib showed multiple wavelet reentry and was resistant to complete suppression by antiarrhythmic drugs or by atrial compartmentalization cryoablation with our protocols. These observations point toward the current clinical dilemma for the treatment of human AFib. Compared to the previous reports, the present swine model showed similar histological and electrophysiological

characteristics to those seen in the mongrel dog[10] or the goat AFib model[9]. However, the success rate (91%) of induction of sustained AFib in our pig model was at least as good as the goat model (88%)[9], but much better than the mongrel dog model (i.e. 20%)[10, 11]. In practice, the atrial dimensions in goats and pigs[17, 18] are bigger to accommodate comprehensive mapping or interventional procedures.

As to the electrophysiological mechanisms, our pig model of sustained AFib provides a better replication of clinical AFib in humans than that in Langendorff-perfused and acetylcholine-facilitated isolated heart preparations[12, 19, 20], especially in terms of providing the integrity for the autonomic nervous inputs. Variations in the activities of either sympathetic, parasympathetic or both nervous systems have been suggested to be crucial for the initiation or maintenance of paroxysmal or chronic AFib in humans[21, 22]. On the other hand, high-dose acetylcholine perfusion has been demonstrated to provoke atrial tachyarrhythmias with a more focal mechanism[19, 20], which may not be desirable for the study of multiple reentrant wavelet activations in common human AFib.

Therapeutic intervention.

With the sustained AFib induced in pigs, we could readily investigate in vivo the therapeutic efficacy and responsible electrophysiologic mechanism. Our study demonstrated a reduction of reentrant wavelets in both right and left atrium after intravenous propafenone, but not dl-sotalol, which might explain a better conversion rate

for propafenone. The effect of propafenone on sustained AFib in our pig model was compatible with the acute efficacy of flecainide and propafenone[23], both I-C drugs, for the conversion of paroxysmal AFib in patients with structurally normal hearts. On the other hand, the demonstration of persistent AFib with independent organized electrical wavefronts inside the compartmentalized right atrial free wall and/or left atrial appendage after linear cryoablation indicated the insufficiency of our ablation protocol for elimination of sustained AFib. Several recent studies by means of surgery[24-26] or catheter ablation[4, 7, 8] have showed the crucial role of the left atrial posterior wall and/or the pulmonary veins in the triggering as well as the maintenance of chronic AFib. Whether or not a different ablation protocol (e.g. left posterior atrial compartmentalization or pulmonary vein encircling) would help eliminating AFib still needs to be confirmed.

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