行政院國家科學委員會專題研究計畫　期中進度報告

在豬隻心房顫動模式中功能性基因體的變化(1/3)

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計畫主持人：賴凌平

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中　華　民　國　92　年　5　月　15　日

行政院國家科學委員會專題研究計畫成果報告

在豬隻心房顫動模式中功能性基因體的變化

Functional Genomic Studies in a Porcine Model of Atrial Fibrillation

計畫編號：NSC 91-2314-B-002-273

執行期限：91 年 8 月 1 日至 92 年 7 月 31 日

主持人：賴凌平　執行機構及單位名稱: 台大醫學院內科

一、中文摘要

本研究計劃有兩個主要目的：

1. 建立在豬隻的心房顫動動物模式
2. 以功能性基因體的觀點，探討心房顫動過程中心房組織的變化

本計劃是一個三年期的計劃，在民國九十一年八月至今我們完成了下列的部份

1. 我們以每分鐘 600 次的速率刺激右心房，成功使這些豬在四週後產生持續性心房顫動。此心、房顫動為永不休止直到豬隻犧牲為止
2. 我們成功使用 DNA 微陣列的方式，大規模篩選各種基因表達的變化
3. 而對於蛋白質的變化，我們成功以二維蛋白電泳的方式，分離許多不同心房蛋白，包括已知和未知的蛋白。

在未來的兩年內我們將預期可以完成下列各項

1. 繼續在豬隻身上製造心房顫動，並使用植入節律器，但不發動刺激的豬作為對照組。而在第 2,4,6 週將豬隻犧牲，採取心房組織，偵測其中的變化。
2. 對於訊息核醣核酸，我們採用 DNA 微陣列的方式，以期大規模篩選各種基因表達的變化。
3. 我們將以二維蛋白電泳的方式，透過心房顫動組與對照組的比較，找出在不同時段有變化的蛋白。
4. 透過訊息核醣核酸及蛋白質變化的互相比對及互相確認，我們將找出有意義的變化並以北方及西方墨點法再加以確認。

本計劃應用功能性基因體之觀點，而以豬隻心房顫動的模型為材料，相信對心房顫動組織的變化能提供前瞻性的突破。

關鍵詞：心房顫動、微陣列、二維蛋白電泳

ABSTRACT

There are two specific aims in the present study:

1. To establish a porcine model of atrial fibrillation.
2. To study the changes of atrial tissue from a viewpoint of function genomics by2-D PAGE and DNA microarray techniques.

This project is a three-year project. During the period between August 2002 and now, we have already accomplished the followings

1. To establish a porcine model of atrial fibrillation by rapid atrial pacing. The atrial fibrillation was observed after rapid pacing for 4 weeks and was incessant until sacrifice.
2. We have established the techniques of cDNA microarray to profile the gene expression pattern in porcine atrial tissue.
3. We have successfully used the 2D-PAGE techniques to separate protein spots in atrial tissue. These protein spot include both known and unknown protein spots.

In the future two years, we will accomplish the following items

1. We will continue the porcine atrial fibrillation model and produce more pigs for mRNA and protein experiment. We will also produce sham pigs by implanting the pacemaker without pacing.
2. We will use the cDNA microarray technique to compare the difference between the gene expression pattern between atrial fibrillation and control pigs.
3. We will use the 2D-PAGE technique to compare the protein spot pattern between atrial fibrillation and control pigs.
4. We will compare the mRNA change and protein change. Significant findings will be re-confirm by Northern and Western blotting techniques

With the present study, we expect to identify crucial changes in the development of atrial fibrillation. This information will contribute to future development of treatment for atrial fibrillation.

Keywords: atrial fibrillation, microarray, 2D-PAGEl

SPECIFIC AIMS

There are two specific aims in the present study

1. To establish a porcine model of atrial fibrillation.
2. To study the changes of atrial tissue in atrial fibrillation from a functional genomic viewpoint using 2-D PAGE and DNA microarray techniques.

BACKGROUND

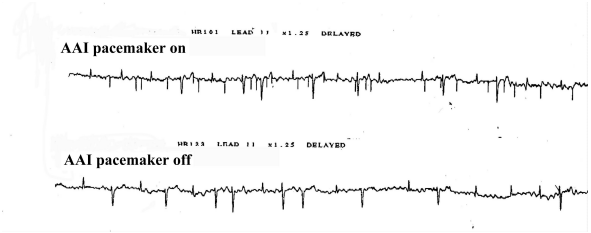
Atrial fibrillation is the most common type of sustained tachyarrhythmia in humans and is responsible for lots of morbidity and mortality as demonstrated in several epidemiological studies.[1-3] Clinically, atrial fibrillation has a tendency to perpetuate itself. Despite vigorous treatment, atrial fibrillation progresses from paroxysmal to persistent. Finally, permanent atrial fibrillation develops and is refractory to all treatment modalities including DC shock. The mechaninsms for the process of so called “atrial fibrillation begets atrial fibrillation” are the focus of many recent studies. Allessie and co-workers reported that atrial electrophysiological properties are changed.[4] They found a shortening of atrial action potential duration. Changes in ionic currents were also reported.[5] Changes in histology and organelles have also been reported.[6] In our own laboratory, we have reported the changes of calcium handling proteins in atrial fibrillation.[7] We also have investigated the changes of potassium channels and funny current channel.[8-9] We are currently establishing the porcine model and it is promising. Our previous studies were dealing with the change of mRNA because the difficulty in getting human tissue. Now, we are shifting our research efforts toward animal model (porcine model of atrial fibrillation). With more atrial tissue available,

we can further investigate the changes in protein level. We can also design to study the time course of various changes, which is not feasible in human studies. With traditional approaches, the changes of several genes or proteins can be studied. Furthermore, previous studies are mostly performed at the late stage of atrial fibrillation. Studies regarding the time course of the changes are hardly available. Therefore, we propose to establish a porcine model of atrial fibrillation. With this animal model, atrial tissues at different stages of atrial fibrillation are available. A genome-wide search for changes at mRNA level and protein level will be analyzed using DNA microarray[10] and 2D-PAGE[11-12] techniques.

PRELIMINARY RESULTS

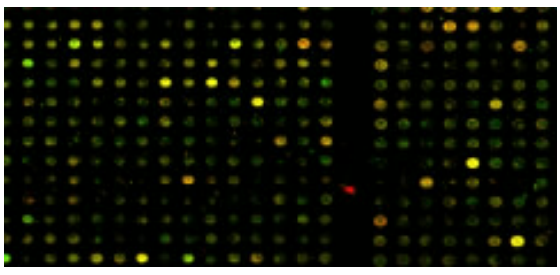
Porcine model of atrial fibrillation

We have performed pacemaker implantation in several pigs. We successfully induced atrial fibrillation in pigs. The pacemaker was turned off after 4 week's pacing and the pig remained in atrial fibrillation for more than 2 weeks. As shown in the following ECG tracing



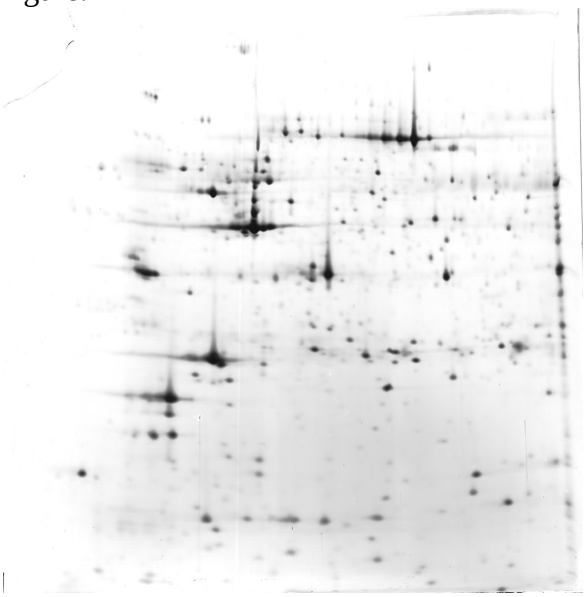
cDNA microarray experiments

To profile the gene expression patterns in porcine atrial tissue, it will be the best to use a porcine cDNA microarray. Unfortunately, such microarray is not available yet. Therefore, we use a human cDNA microarray containing 6055 genes. After adjusting the washing stringency, satisfactory results were obtained and a representative picture is shown below



2D-PAGE

With limited resources, we have performed a 2-D PAGE using the atrial tissue from a pig with atrial fibrillation. We have adjusted the conditions have got some preliminary data as shown in the following figure.



FUTURE WORKS

Second year

1. We will continue on producing pigs with atrial fibrillation as well as control pigs.
2. We will continue the work with 2-D PAGE
3. More DNA microarray studies will be carried out.
4. Comparison among different groups will be done.

Third year

1. We will complete the animal part of the study and collect enough number of atrial samples from all different groups of pigs.
2. We will collecting all data and identify

the protein spots and mRNA with significant changes.

3. The nature of the protein spots with significant changes will be studied.
4. We will prepare the data and write the manuscript for publication.

EXPECTED RESULTS AND POSSIBLE CONTRIBUTIONS

1. The porcine model is unique in the world. The size of porcine heart is very similar to that of human heart. The baseline heart rate of pigs is also similar to human heart rate.
2. We can provide information about the time sequence of various changes. Past studies are usually performed in chronic atrial fibrillation and detect changes in late stage.
3. DNA microarray can provide information about gene expression in a scale of thousands.
4. 2-D PAGE can separate about 2000 proteins including known and unknown ones. This is a good way to identify changes of novel proteins.

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