

## Original Article

# Application of the myocardial tissue/silicon substrate microelectrode array technology on detecting the effect of Zhigancao Decoction medicated serum on cardiac electrophysiology

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Received September 27, 2014; Accepted January 21, 2015; Epub February 15, 2015; Published February 28, 2015

**Abstract:** Background: Modern pharmacological studies have confirmed that the total extract of Zhigancao Decoction, either as a single active compound or in combination, can inhibit arrhythmia. In this study, the myocardial tissue/silicon substrate microelectrode array (MEA) was used to detect the Zhigancao Decoction medicated serum of the New Zealand white rabbits right atrial appendage after rapid right atrium pacing (RAP). Methods: New Zealand white rabbits were randomly divided into four groups, with eight rabbits per group. The first group was the control animal group (Group A). The second was the drug-free serum vehicle control group (Group B). The third group used serum-containing f Zhigancao Decoction (Group C) at various concentrations. The fourth group was the Zhigancao Decoction medicated serum group (Group D). After establishing the atrial fibrillation model, the field action potential duration (fAPD) of the right atrial appendage (RAA) in the control group, and in groups after different interventions, were measured. Results: We report of an atrial fibrillation model using by rapid right atrium atrial pacing, in which fAPD was significantly shorter 12 hours after pacing ( $P < 0.05$ ). The intervention by 10% to 25% of drug-containing serum or decoction could prolong the fAPD of rabbit atrial appendage in atrial fibrillation rabbits in a dose dependent manner ( $P < 0.05$ ). Conclusion: fAPD can be used as an indicator for the change of cardiac electrophysiological properties. 10% to 25% of Zhigancao Decoction medicated serum can prolong fAPD in atrial fibrillation rabbits, which may be the electrophysiological mechanism of atrial fibrillation resistance.

**Keywords:** Rapid pacing the right atrium, atrial fibrillation, microelectrode array, Zhigancao Decoction, drug containing serum

## Introduction

Atrial fibrillation (AF) is one of the most common cardiac arrhythmias (AF), with a population incidence rate of 1% that increases with age. Patients have a significantly impaired quality of life and increased likelihood of morbidity and mortality [1]. The Specialized Committee of Diagnostics of Traditional Chinese Medicine, China Association of Chinese Medicine, has denominated the disease as a heart palpitate [2].

Long-term clinical practice has demonstrated that Zhigancao Decoction is an effective therapeutic agent in managing AF. The therapeutic

effect of Zhigancao Decoction medicated serum is comparable to routinely used anti-arrhythmic medicines. Moreover, this compound also has advantages in improving many associated symptoms, with no significant side effects observed. Long-term use in patients has also demonstrated good tolerance to the drug in patients.

Extensive pharmacological research of ginseng decoction is reported in the literature, with a strong emphasis on measuring the effects of crude extracts or specific active ingredients. The serum pharmacological method has been widely used to study the mechanism of Chinese medicines, as demonstrated in

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organ tissue culture [3], cell culture, and gene expression [4].

In this study, we used the serum pharmacology method, in combination with electrophysiological techniques, to examine the therapeutic effect of serum-containing Zhigancao Decoction in the treatment of atrial fibrillation. This data lays a theoretical basis for additional studies examining the effect of traditional Chinese medicines on the cardiac ion channels.

### Materials and methods

#### *Experimental animals and grouping*

32 adult New Zealand white rabbits, weighing of 2.5-3 kg, male or female, were provided by the Experimental Animal Center of Xinjiang Medical University (License No.: SCXK (Xinjiang) 2003-001). They were randomly divided into four groups, eight for each group: control group (A) with perfusion in the right atrial appendage but without rapid pacing; normal serum group (group B) with perfusion of normal serum in the right atrial appendage 12 hours after the short-term rapid pacing in the right atrium; Zhigancao Decoction medicated serum group (group C) with perfusion of Zhigancao Decoction medicated serum in the right atrial appendage 12 hours after the short-term rapid pacing in the right atrium; and Zhigancao Decoction group (group D) with perfusion of Zhigancao Decoction in the right atrial appendage 12 hours after the short-term rapid pacing in the right atrium.

#### *Preparation of drugs*

Zhigancao Decoction was prepared as previous described [5]. Briefly, licorice (20 g), ginger (15 g), ginseng (10 g), habitat (80 g), cassia twig (15 g), gelatin (molten; 15 g), radix (10 g), maren (10 g), and nine jujubes were immersed in 1.2 L of cold water (6 ml water for per gram of crude drug) for 30 min. This mixture was then boiled and then slowly fried for 40 min, filtered when hot and emptied by gravity flow. The remaining pellet was then immersed in another 0.8 L of water (4 ml water for per gram of crude drug), boiled and then slowly fried for 40 min, filtered when hot, and emptied again by gravity flow. The filtrates were combined and concentrated to 200 ml at 1 g/ml in 95°C water bath.

Serum-containing fried glycyrrhizae decoction was prepared as described: rabbits were given

the fried glycyrrhizae decoction by gavage twice daily for consecutively 3 d (1 ml/100 g body weight), which was 20 g/kg/d raw medicine and six times of human daily dosage. Before administration of the last drug dose, the rabbits were put through fasting for 12 hours with free access to water. 1 h after the last drug dose, rabbits were anesthetized with chloral hydrate. Carotid artery blood was collected, stored at room temperature for 2 hours in sterile conditions and at 4°C overnight. After that, the blood was centrifuged at 2,500 r/min for 10 min to separate serum, which was further sub-packaged and deactivated at 56°C for 30 min and stored at 70°C before use [6].

As a result, mice were prepared using normal serum: the rabbits were given distilled water by gavage twice daily for consecutively 3 d by 1 ml/100 g body weight, which was 20 g/kg/d raw medicine and six times of human daily dosage. Before being given the last drug dose, the rabbits were put through fasting for 12 hours. The blood was collected 1 hour after the last drug dose and serum was separated under aseptic conditions, inactivated at 56°C for 30 min, and stored at 20°C before use.

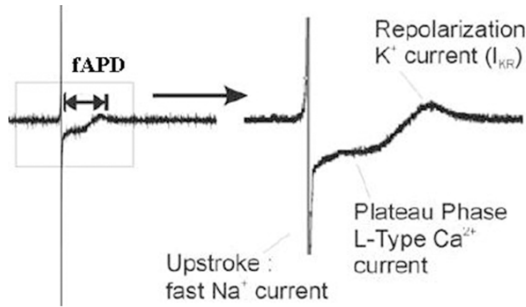
#### *Rapid atrial pacing (RAP) model preparation*

The rabbits were anesthetized through ear vein injection of 30 mg/kg sodium pentobarbital (3% in mass fraction) [7]. They were fixed on the platform, and after endotracheal intubation and ventilator-assisted breathing, neck incision was made; then the right internal jugular vein was separated and cut, ligation at the head-end. Under the guidance of B-mode ultrasound, longitudinal visual was used to assure the precise position of the electrode tip. Then move horizontal to assure that the electrode tip was in the right atrium. The LEAD-2007 electrophysiology instrument (Jinjiang Electronic Inc., Sichuan, China) was used to give continuous single stimulus for 24 hours of RAP, with a pacing rate of 600 beats/min, a pulse width of 0.5 ms, and an intensity of 2 V. Sham group was also implanted with pacing electrodes, but without RAP.

#### *Microelectrode Arrays (MEA) in recording the field action potential duration (fAPD)*

At the center of the MEA, there is a 50 mm × 50 mm transparent quartz glass, and at the center of the glass, there was an area of 0.78 mm ×

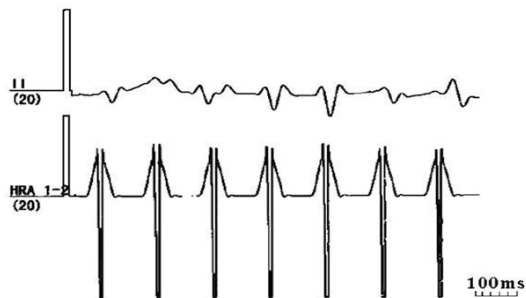
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**Figure 1.** The fAPD and ion current recorded by the MEA.



**Figure 2.** The cardiac electrophysiology at the back of the right atrium and ECG in normal rabbits.



**Figure 3.** The cardiac electrophysiology at the back of the right atrium and ECG in RAP rabbits.

0.78 mm with a total of 60 titanium nitride electrodes ( $8 \times 8$ ); the diameter of the electrode tip was  $10 \mu\text{m}$ , and the spacing between adjacent electrodes was  $100 \mu\text{m}$ . The software MC-Rack was used to record and process the field potentials (FP) parameters: the first negative peak (FPmin), the final positive peak (FPmax), and the field action potential duration (fAPD) (namely, FPdur, the time from FPmin to FPmax), as shown in **Figure 1**. In this study, specimens were fixed in the MEA recording disc and the temperature was maintained at  $37^\circ\text{C}$  by the  $\text{TCO}_2$  temperature controller, and perfused for



**Figure 4.** The right atrial appendage fAPD before and after ARP. A. The right atrial appendage fAPD in normal rabbit; B. the right atrial appendage fAPD in AF rabbit.

30 min at a speed of 3 ml/min with 95%  $\text{O}_2$  + 5%  $\text{CO}_2$  mixed gas. A Stimulus Generator is used to give stimulus in an intensity twice of the threshold, with a pulse width of 2 ms and a frequency of 1 Hz, as the driving pulse. After sample stabilization for 30 min, the fAPD of the right atrial appendage was recorded.

### Statistical analysis

All data were expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ), and analyzed with the SPSS16.0 software. The comparison between two groups was conducted through a comparison of their means, and subjected to t test, while the mean comparison among multiple groups was conducted using one-way ANOVA, followed by LSD test for pairwise comparison. If there was a heterogeneity of variance, the rank sum test was adopted where  $p < 0.05$  is considered statistically significant.

### Results

#### *The cardiac electrophysiology at the back of the right atrium and ECG in RAP rabbits*

12 hours after the RAP in the right atrium, the atrial surface ECG became disordered and there was no P wave in the ECG. Rather, an f-wave was detected with an uneven amplitude and interval. The frequency was 450 to 600 beats/min and the R-R interval was also uneven. These characters would last for more than 10 s, which suggested the successful establishment of the rabbit atrial fibrillation model (**Figures 2 and 3**).

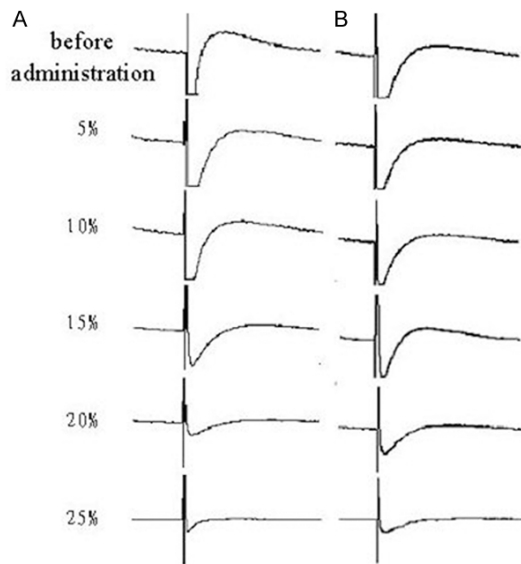
#### *The comparison of the right atrial appendage fAPD before and after ARP*

The right atrial appendage fAPD was significantly shortened from  $174.30 \pm 1.36$  ms to  $162.48 \pm 0.88$  ms after ARP ( $P < 0.05$ ; **Figure 4**).

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**Table 1.** The dose-effective relationship analysis right atrial appendage fAPD among four groups ( $\bar{x} \pm s$ ),  $n = 8$ , ms)

Group	Before administration	After administration				
		5%	10%	15%	20%	25%
A group	174.30 ± 1.36	176.70 ± 1.50	171.50 ± 1.82	175.67 ± 1.59	175.88 ± 2.23	172.51 ± 0.85
B group	162.48 ± 0.88	161.68 ± 1.10	162.00 ± 0.68	160.62 ± 0.45	159.22 ± 1.10	162.26 ± 0.57
C group	163.04 ± 1.18	162.11 ± 0.55	170.81 ± 0.61*	171.00 ± 0.46*	179.08 ± 0.67*.#	179.76 ± 2.26*.#
D group	160.98 ± 0.41	158.82 ± 0.64	163.82 ± 0.78*	163.66 ± 0.95*	174.06 ± 1.32*.#	176.84 ± 1.19*.#



**Figure 5.** The dose-dependent effect of Zhigancao Decoction medicated serum and water-based decoction on the right atrial appendage fAPD in atrial fibrillation rabbits. A. Medicated serum; B. Water decoction.

### The dose-dependent effect on the right atrial appendage fAPD

After being given different concentrations of Zhigancao Decoction within and without serum, the in-group comparison showed that when the concentration reached 10%, the fAPDs were significantly longer in serum and decoction group ( $P < 0.05$ ) than those before these interventions. When the concentration reached 25%, there was no further increase in fAPD, no matter in serum or decoction group, and there was no statistically significant difference (**Table 1; Figure 5**).

### In-group comparison of Zhigancao Decoction medicated serum and water decoction on the fAPD

The inter-group comparison showed that when the concentration reaches 10% to 25%, the

fAPD in drug-containing serum group was significantly longer than that in water decoction group, and the difference was statistically significant ( $P < 0.05$ , **Table 2**).

### Discussion

The origin of Zhigancao Decoction can be found from Article 177 of the 'Treatise on Febrile Diseases' by Zhongjing Zhang from the Eastern Han Dynasty: "Typhoid fever is characterized by irregular pulse and severe palpitation, and usually treated by Zhigancao Decoction". Zhigancao Decoction is a classic Chinese medicine for the treatment of thoracic obstruction [8]. For clinical application, the dose of Zhigancao Decoction depends on individual patient and symptoms. Licorice acid and ginseng are both saponins, which are the active ingredients for anti-arrhythmia [9, 10]. Experiments have demonstrated that Zhigancao injection can antagonize arrhythmias in animals induced by chloroform, adrenaline, aconitine, poison K, and barium chloride [11]. In clinical studies, Hong feng Guan et al describe the use of Zhigancao Decoction in combination with astragalus, peony, salvia, and tinglizi, to strengthen the heart and induce diuresis [12].

In 1987, the Japanese Kampo expert Shinichi Tashiro said in his study about Kampo that animal serum could be used in pharmacological experiments on Kampo formulations, and that this method was pertinent to the characteristics of complex ingredients in Chinese medicine compared to application of the compounds directly *in vitro* [9-15]. First, the serum-containing drug was added into the *in vitro* reaction system in order to avoid interference of non-specific physical and chemical factors in the production process of the drug or crude preparation. Secondly, serum-containing drugs are more stable and easier for *in vitro* administration and detection of drug effects. Thirdly, it is easy to study the mechanism of drug action at the cellular and molecular level, and screen

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**Table 2.** Comparison on right appendage fAPD given different concentrations Zhigancao Decoction containing serum and water decoction among four groups in atrial fibrillation rabbit ( $\bar{x} \pm s$ , n = 8, ms)

Group	10%	15%	20%	25%
C group	170.81 ± 0.61	171.00 ± 0.46	179.08 ± 0.67	179.76 ± 2.26
D group	163.82 ± 0.78*	163.66 ± 0.95*	174.06 ± 1.32*	176.84 ± 1.19*

Note: Comparison with Zhigancao Decoction containing serum, \*P < 0.05.

active ingredients in combination with serum drug chemistry. Therefore, serum-containing drug application is a more efficacious approach to studying the effects of traditional Chinese medicines [16, 17].

This method is currently a hotspot in pharmacology research, and is also a link of modern science and technology with traditional medicine. Some domestic electrophysiological studies have shown that Zhigancao Decoction medicated serum inhibits intracellular calcium ion channels in rabbit in a dose-dependent manner [18]. This may be a possible mechanism of action in the treatment of arrhythmia by frid glycyrrhizae decoction.

In this study, the cardiac electrophysiology technology was used to establish the right atrial fibrillation model, verified by ECG and cardiac electrophysiology. The MEA technology was used to detect the fAPD in the right atrial appendage. The results showed that the fAPD was significantly shortened after pacing. Combined with changes in cardiac electrophysiology, these results suggest that fAPD could be used as an effective indicator of cardiac electrophysiological properties changes, especially in drug screening study of anti-arrhythmics.

Qinghua Ma, et al. found that Zhigancao Decoction had a good ability to reduce peripheral TNF- $\alpha$  in rat AF model via inhibiting the expression of inflammatory factors. These eventually ameliorate tissue damage from atrial fibrillation [19]. Lanying Chen, et al. also found that Zhigancao Decoction could significantly strengthen the immune system in rats, ameliorate arrhythmia in a rat model of blood deficiency [20]. Jiunue Hu et al. treated different arrhythmias rat models with Zhigancao Decoction and found that treatment could significantly delay stroke chamber, ventricular tachycardia, ventricular fibrillation and time of death caused by aconitine and calcium chlo-

ride, and reduce the period of arrhythmia [21].

This study is based on the myocardial tissue/silicon substrate microelectrode array technology and explored the effect of Zhigancao Decoction medicated serum on cardiac electrophysiology. After treat-

ment with different concentrations of serum-containing decoction, the fAPDs in the right atrial appendage of rabbits were extended. According to the results, a dose-dependent relationship was observed showing that 10% to 25% of serum-containing decoction had an effect on the fAPD of the right atrial appendage. When the drug concentration reached 25%, no further increase of efficiency was found, suggesting that 25% of drug was the maximum effective concentration for Zhigancao Decoction medicated serum and water-based decoction. The inter-group fAPD differences in the effects of 10% to 25% serum and decoction were also compared. The results suggested that the effect of 10% to 25% decoction containing serum on fAPD was better than that treatment with decoction in water. In addition, the results suggested that the physical and chemical properties, such as impurities, pH, and osmotic pressure, of crude extracts of Zhigancao Decoction were not constant and interfered within this *in vitro* study.

In recent years, biosensors have been developing rapidly and have extended beyond the limits of traditional biosensors represented by enzyme electrodes. New high-affinity biosensors have emerged that can continuously transfer real-time information of receptor-ligand binding. At present, cells and tissues from different parts of the heart can be fixed on the surface of MEA and other silicon devices, resulting in myocardial cell chip constituting myocardial cells coupled with semiconductor silicon substrate chip material, by which myocardial cells and metal micro-electrodes have formed one-to-one relationship. It can non-destructively transfer the information of resting potential and action potential signals of cardiomyocytes to the peripheral circuits, achieving the real-time measurement of electrophysiological activities. Compared with patch-clamp and microarray technologies, it can simultaneously



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measure the action potential in different locations, to achieve coupling measurement among cells, and non-destructively and simultaneously record multiple action potentials in excitable cells or tissues. Cardiomyocyte chips can be used not only in electrophysiological studies on cardiomyocytes, but also in studies on the communication among cells and the activities of cardiac tissue sections, as well as multi-point measurements of tissue slices and screenings of drugs or toxic substances.

The results suggested that, fAPD corresponded with the duration of  $Ca^{2+}$  and  $K^+$  ion channels. We hypothesize that Zhigancao Decoction medicated serum reduces the incidence and maintenance of atrial fibrillation possibly via acting on  $Ca^{2+}$  and  $K^+$  channels. Ionic mechanism on the Zhigancao Decoction medicated serum in anti-arrhythmic has not been reported previously. In this study, the microelectrode chip technology was used to study the effect of Zhigancao Decoction medicated serum on electrophysiological properties, which helped to explore the drug target in treatment of AF by traditional Chinese medicine.

### Acknowledgements

This work was supported by grant 2013911119 from Science and Technology Supporting Project of Xinjiang Uygur Autonomous Region.

### Disclosure of conflict of interest

None.

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