Experimental Evaluation of Myocardial Fibrosis in a Rapid Atrial Pacing Model in New Zealand Rabbits using Quantitative Analysis of Ultrasonic Backscatter

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABDEF 1 Juan Sun*
ABDF 2 Zhang Rong*
ABDEF 3 Najina Wugeti
ABDEFG 3 Adila Azhati
ABDF 3 Yujun Guo
ABDF 3 Haili Liu
ABDF 3 Ruping Qian
ABDF 3 Long Zhao
ABDF 3 Yitong Ma

1 Clinical Postdoctoral Research Station, The First Affiliated Hospital of Xinjiang Medical University, Urumuqi, Xinjiang, China
2 Function Center, Xinjiang Medical University, Urumuqi, Xinjiang, China
3 Heart Center, The First Affiliated Hospital of Xinjiang Medical University, Urumuqi, Xinjiang, China

* Contributed equally to this work
Corresponding Author: Yitong Ma, e-mail: myt-xj@163.com
Source of support: This work was supported by grant 81360043 from the National Nature Science Foundation of China

Background: The aim of this study was the establishment of a rapid atrial pacing (RAP)-induced atrial fibrillation (AF) model with electrophotoluminescence and the application of ultrasonic backscatter quantitative analysis of the degree of myocardial fibrosis in New Zealand white rabbits.

Material/Methods: Sixteen New Zealand white rabbits were randomly divided into 2 groups: 1) a sham operation group (n=8) with implanted electrodes and no rapid pacing and 2) a pacing group (n=8) with an AF model induced by short-term rapid right atrial pacing for 12 h. Establishment of an AF model, atrial myocardium of myocardial fibrosis was tested by Masson staining and expression of collagen I and collagen III protein was detected with pathologic immunohistochemistry integrated back-scatter (IBS). Back scattering integral cycle variation (CVIB) were detected in atrial septal and posterior wall of the right atrium.

Results: Rapid atrial pacing successfully induced the atrial fibrillation model in rabbits. Masson staining showed myocardial fibrosis significantly increased in the pacing group. Expression of collagen I and collagen III protein was strongly positive in the pacing group, and expression of collagen I and collagen III protein were weakly positive in the sham operation group. Compared with the sham operation group, AII was increased (8.24±0.85 *vs.* 15.56±1.30, P<0.05) and (7.58±0.56 *vs.* 16.60±2.45, P<0.05). CVIB was significantly decreased (2.78±0.86 *vs.* 1.08±0.13, P<0.05) and (3.12±0.65 *vs.* 1.56±0.15, P<0.05) in septal and posterior wall of the right atrium of the pacing group.

Conclusions: Ultrasonic backscatter measurement technique can be used to evaluate degree of myocardial fibrosis in a right atrial pacing-induced atrial arrhythmia model.

MeSH Keywords: Atrial Fibrillation • Atrial Remodeling • Cardiac Pacing, Artificial • Ultrasonography

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/891242

2104   1   5   15

Background

Atrial fibrillation (AF) is one of the most common and complicated arrhythmias in clinical practice. Some researchers have reported that the prevalence rate of AF is 0.77% and in China it reaches 7.5% in the population over age 80 [1]. At present, the pathological and physiological mechanism of AF it is still unclear. It is now recognized that the atrial electrical remodeling of AF has an essential relation to the occurrence and duration of AF. Recently, research outside China has shown that the remodeling of the basic atrial structures may occur before, during, or after cardiac electrical remodeling. This remodeling of the structures is important in the development of continuing AF, in which the structural remodeling presents as atrial expansion and fibrosis on a macro level. In addition, studies suggest that atrial fibrosis is a structural basis of occurrence of AF and that myocardial fibrosis is the result of unbalanced collagen synthesis metabolism and degradation metabolism, which has a very complex mechanism in which multiple systems participate in the adjustment of collagen metabolism [2,3]. In the course of atrial fibrosis, the changes in conductivity of some local atrial tissues can promote the occurrence and maintenance of atrial fibrillation and then the suppression of atrial fibrosis can reduce the incidence of AF [4,5]. With the rapid development of ultrasonic technology, assessment of left atrial function is constantly progressing. The hemodynamics may indirectly reflect the functional state of the left atrium as revealed by Doppler technology. In recent years, strain rate imaging, speckle tracking technology, and acoustic quantification technique can directly display left atrial function. Speckle tracking echocardiography can accurately and objectively evaluate and identify the different pathological conditions of left atrial function. Because this technology is not based on Doppler imaging but instead is based on 2-dimensional ultrasound, it has no angular dependence and measures atrial local strain [6]. Strain rate imaging and speckle tracking technology are limited to 1 axis and only show local myocardial function of the left atrium, but the local anomaly in myocardial function does not represent the overall function of the heart. Acoustic quantification technique tracks left atrial volume change. Using the difference between myocardial tissue and blood backscattering signal length, it is mainly based on the ultrasonic backscattering integral principle to discern and track the boundary between the 2, thus displaying area and volume of the left atrium. In combination with left atrial characteristics, it accurately reflects left atrial function. The present study established AF model induced by rapid pacing of the right atrium of rabbits and made a quantitative analysis of the degree of myocardial fibrosis of atrial remodeling under rapid atrial arrhythmias with ultrasonic backscatter technology.

Material and Methods

Experimental animals and grouping

A mixed-sex group of 16 adult New Zealand white rabbits weighing 2.5~3kg was provided by the Experimental Animal Center of Xinjiang Medical University. The rabbits were of first-class quality (License No.: SCXK (Xin) 2003-001). The study conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Publication No. 85-23, revised 1996). These animals were divided into 2 groups: 1) a sham operation group in which pacing electrodes were implanted without rapid pacing and 2) a pacing group in which animals received a short period of rapid pacing to the right atrial for 12 h and an AF model was induced. There were 8 rabbits in each of the 2 groups.

Preparation of rapid atrial pacing (RAP) model [7]

After being anaesthetized through vein injection along the edges of ears with 30 mg/kg of 3% sodium pentobarbital, the rabbits were positioned face upwards. Then the pipes were inserted into the trachea and a respirator was used to facilitate breathing. An incision was made on the right side of the neck. The inside veins were was separated and opened with ligation of the end near the head. Under the direction of B-type scanning, positioning was conducted with B-type scanning guidance longitudinal cutting, keeping the B-type scanning parallel with the electrode. Longitudinal observation was made to ensure the correct positioning of the electrode tip, then cross-cutting observation under B-type scanning was made to see that the electrode tip was inside the right atrium. An electrical physiological apparatus, the LEAD-2007 (Sichuan Jinjiang Electronics), was used to conduct continuous single-stimulation mode for 12 h of RAP, with the pacing frequency of 600 times/min, width of pulse at 0.5 ms, and strength of 2 V. The sham operation group only had the pacing electrode implanted and received no high-level rapid pacing stimulation.

Collection and treatment of acoustic densitometry (AD) data [7]

AD-IBS software configured with an HP Sonos-5500 ultrasonic diagnostic device. At the time of measurement, the section of the long axis of the left ventricle beside the breastbones was taken. The ultrasonic setting was adjusted into AD state, IBS sampling, and analysis status. The region of interest was set into sampling frames of 21×21 pixels and then separately placed into the atrial septum and rear wall of the right atrium. According to the results of image analysis, the instrument can automatically determine the acoustic densitometry index – the integrated backscatter value (IBS) and cyclic variation of integrated backscatter (CVIB) values, as well as average

Figure 1. The new zeland rabbit normal electrocardiograms recorded recorded by electrophysiologic and body surface electrocardiogram.

Figure 2. The new zeland rabbit electrocardiograms of the right atrial pacing recorded by electrophysiologic and body surface electrocardiogram.

image intensity (AII) value of the atrial septal and rear wall of the right atrium.
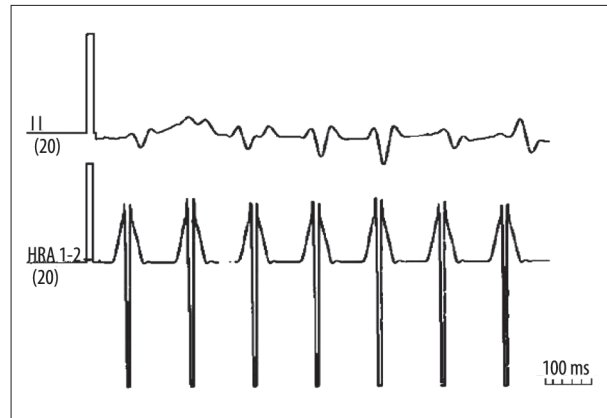
Masson staining

Masson staining consisted of: (1) Regular dewaxing of slices; (2) compound staining fluid Masson for 5 min; (3) slight wash with 0.2% acetic acid aqueous solution; (4) 5% phosphate-tungstic acid for 5–10 min; (5) thorough cleaning twice with 0.2% acetic aqueous solution; light staining fluid for 5 min; 0.2% acetic aqueous solution wash twice; and (6) anhydrous acetic acid dehydration; transparent xylene; sealing with neutral gum.

Detection of protein expression of I-type collagen and III-type collagen

The auricular dextra tissue was embedded with paraffin after fixation, with coronal surface of slices at a thickness of 4 µm. Then the specimen was dewaxed and heat rehabilitation of antigen under high pressure was made, followed by dyeing to collagen I and collagen III using the SP immunohistochemistry staining method. Comparison in negative was conducted to use PBS instead of primary antibodies. The color of collagen I and collagen III was represented in that the cytoplasm was dyed brownish yellow, which showed to a positive result. The coloration was divided in terms of degree, including: colorless for negative, light brownish yellow for positive, and brown for strong positive.

Statistical methods

SPSS 16.0 software was used in statistical analysis. Data are given as means ± standard deviation. The comparison between the 2 groups used The t-test designed separately with complete randomization, with $P < 0.05$ as showing a statistically significant difference.

Results

Changes in intracardiac electrophysiology diagram and surface electrocardiogram after pacing the right atrium

After 12-h pacing of the right atrium, there are disorderly electrical activities in the atrial surface electrocardiogram, in which the representation of the electrocardiography diagram disappeared and, instead, wave-f occurred of different sizes, uneven spacing, and various forms, with frequency of 450~600 times/min, lasting for more than 10 s. And then the AF model of rabbits succeeded (Figures 1 and 2).

### Masson dyeing of auricula dextra tissue of the 2 groups

In the sham operation group, through the dyeing with Masson, a few collagenous fibers become blue, cardiac muscle fiber become red, and the collagenous fiber combined with each other to form a network shape (Figure 3). In the pacing group, the results of dyeing with Masson show that much collagenous fiber appeared blue-green and the network was distributed between the red cardiac fibers. Moreover, the destruction of collagenous fiber was disorderly, with the cardiac bundles twisted and separated by many collagenous fibers (Figure 3).

Protein expression of collagen I and collagen III of auricular dextra tissue of the 2 groups

In the sham operation group, the expression of brown proteins of collagen I and collagen III among myocardial cells can be seen as weakly positive. In pacing group, the expression of tan-colored proteins of collagen I and collagen III among the cardiac cells showed as strongly positive (Figures 4 and 5).
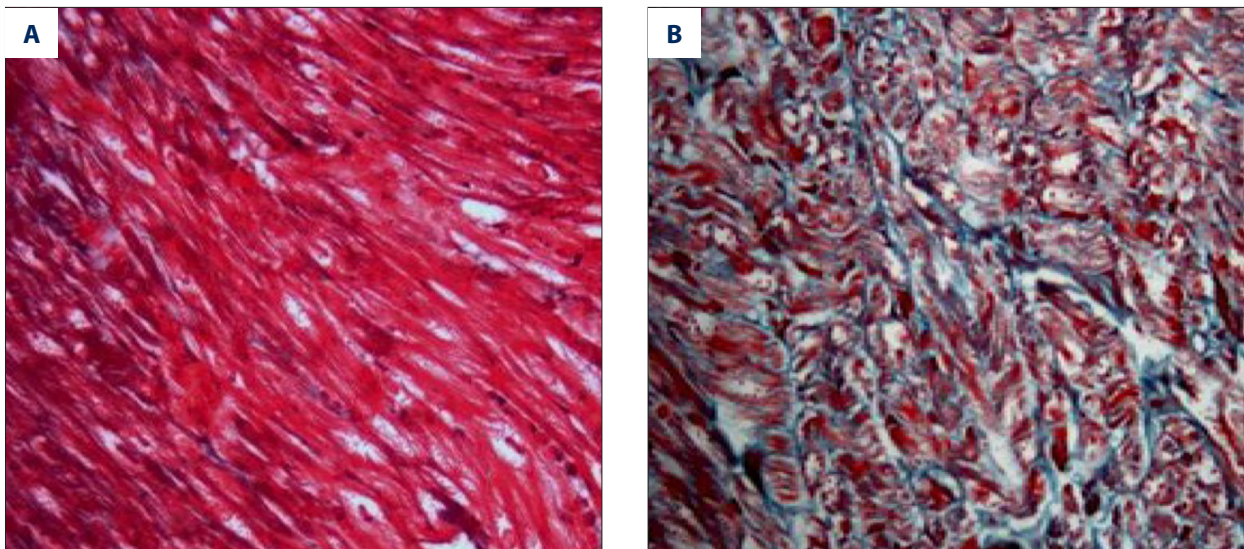
Figure 3. Pathology slides of fibrosis in the right auricular tissue from both groups of rabbits (Masson, ×400). (**A**) Shows a small quantity of collagenous fibers blue in the sham-operated group; (**B**) shows a large quantity of collagenous fibers and stained bluish green in the rapid atrial pacing group.
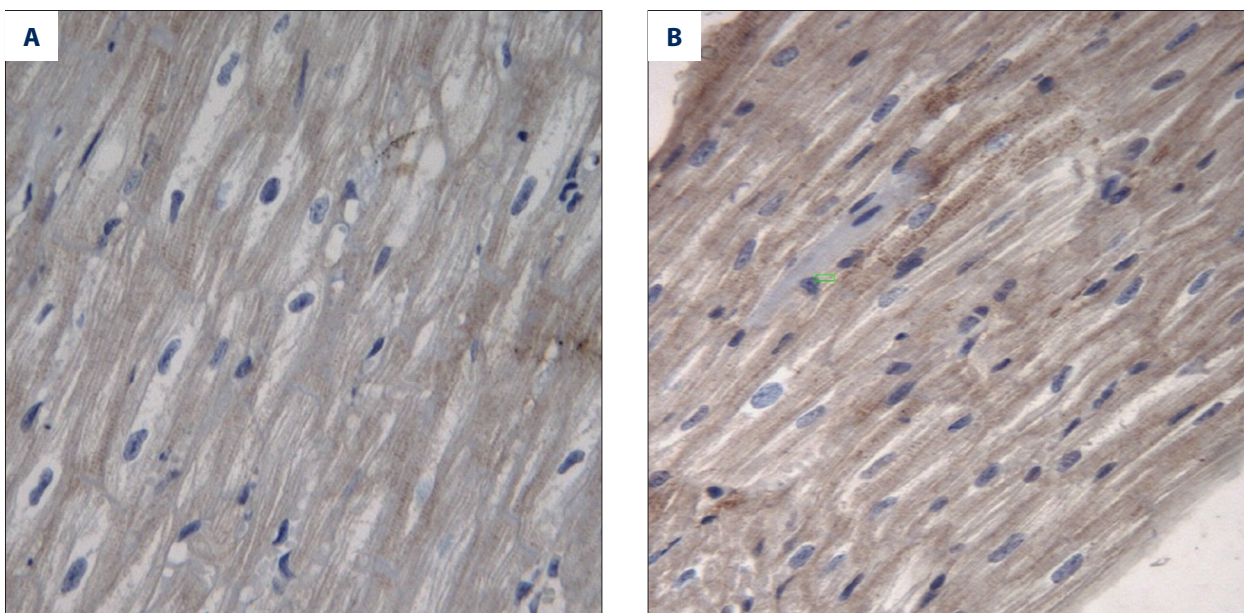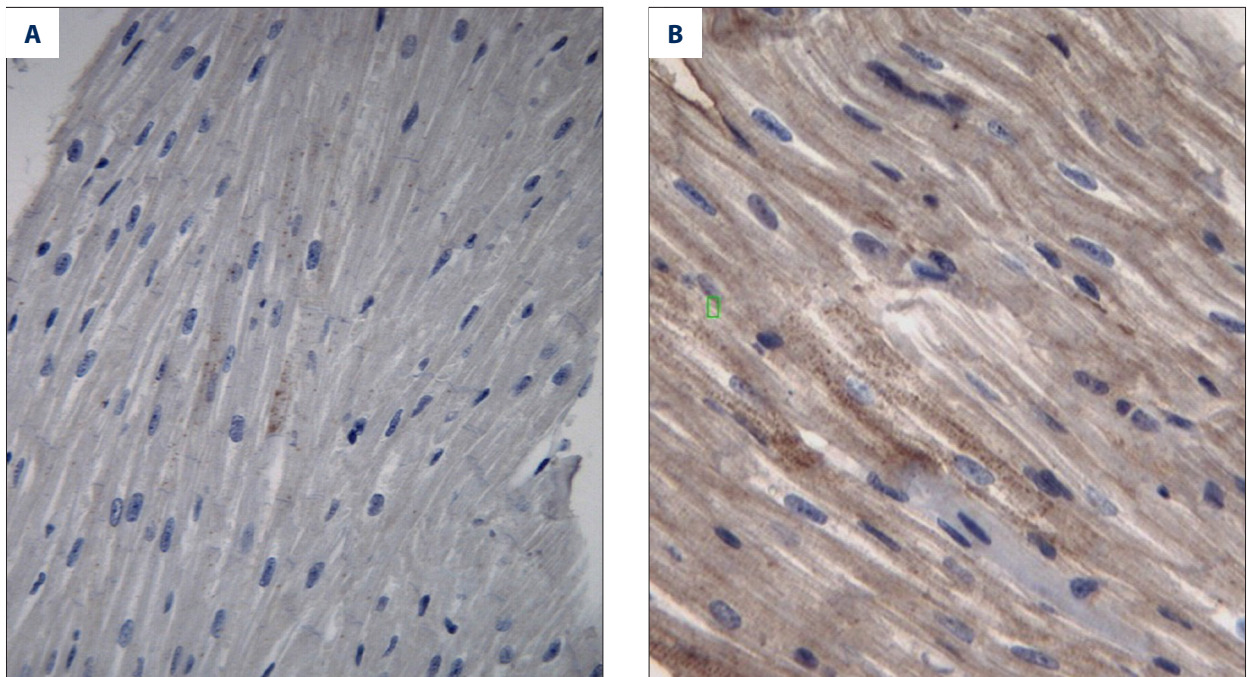

Figure 4. Immunohistochemistry of collagen I protein in the right auricular tissue from both groups of rabbits (SP, ×400). (**A**) Shows weakly positive collagen I expression in the sham-operated group; (**B**) shows strongly positive collagen I expression in the rapid atrial pacing group.

Comparison of indicator AD of the 2 groups

Compared with the sham operation group, the AII of the interatrial septum and the rear wall of the right atrium was remarkably increased in the pacing group, and the difference had statistical significance (P<0.05). In addition, CVIB of the interatrial septum in the pacing group was clearly reduced compared with that in the sham operation group, and the difference had statistical significance (P<0.05) (Table 1).

Discussion

When atrial electrical remodeling returns back to normal, the underlying reasons for persistent AF and structural remodeling are still present. Thus, atrial electrical remodeling alone is sufficient to induce AF, while structural remodeling is responsible for maintaining AF [9,10]. In this study, we have successfully established the electrical stimulation rapid pacing right atrium-induced AF model. Cardiac ultrasound technology was

Figure 5. Immunohistochemistry of collagen III protein in the right auricular tissue from both groups of rabbits (SP, ×400). (**A**) Shows weakly positive collagen III expression in the sham-operated group; (**B**) shows strongly positive collagen III expression in the rapid atrial pacing group.

Table 1. Comparion of AII and CVIB in two groups (n=8, $\overline{\chi}\pm s$).

Groups	AII		CVIB	
	Atrial septal	The posterior wall of the right atrium	Atrial septal	The posterior wall of the right atrium
Sham operation group	8.24±0.85	7.58±0.56	2.78±0.86	3.12±0.65
Pacing group	15.56±1.30*	16.60±2.45*	1.08±0.13*	1.56±0.15*

Compared with Sham operation group, * P<0.05.

applied to evaluate the extent of myocardial fibrosis. Our results show that myocardial fibrosis appears after pacing the rabbits' atrium for 12 hours.

The normal myocardial interstitium consists of cellular structures and non-cellular structures in which over 90% of the main element of the interstitium consisted of collagen I and collagen III. The components of various collagens jointly formulate the collagen fiber network, combining the myocardial cells into a whole body, which has a direct influence on heart structure and function. The myocardial fibrosis was mainly characterized by increased deposits of collagens in the interstitium, unbalanced ratios of various collagens, and disorderly arrangements. The insulating property of extracellular matrix ensures the conductivity of cardiomotility along the correct paths. This suggested that the homogeneous conductivity of the electrical activity of the atrium not only depends on the synchronous activities of the myocardial cells, but also has relations to the mutual action between extracellular matrix and myocardial cells in the course of conductivity [11]. Chrsostomakis et al. maintained the continuous occurrence of atrial fibrillation by means of paroxysmal rapid pacing, which showed that rapid atrial arrhythmia stimulates formation of myocardial fibrosis [12]. Based on the establishment of an AF model induced by rapid atrial pacing, this experiment detected the degree of atrial cellular fibrosis. The study shows that many collagen fibers had a disorderly distribution after Masson dyeing of the atrial cells in the pacing group. Protein expression of collagen I and collagen III were strongly positive, and the expression of the 2 types of collagen increases to further reduce the adaptability of atrial muscles. AF can induce atrial myocardial fibrosis, and, in turn, the atrial structural remodeling is the structural basis for the maintenance of AF, in which the 2 interact as both cause and effect, forming a vicious cycle.

With the new non-invasive ultrasonic characterization technology developed in recent years, ultrasonic backscatter technology can make a quantitative evaluation of the properties and functional status of tissues and organs. The ultrasonic characterization of myocardial tissue is based on the theory that it will clearly show changes in the properties of myocardial physics (acoustics) and changes in myocardial pathology, and thus the patterns and degrees of myocardial pathological changes can be evaluated and analyzed through the detection of the changes in IBS parameters [13]. In the present experiment, the ultrasonic backscatter determination technology was applied to measure the degree of myocardial fibrosis in atrial arrhythmia. The results showed that the AII of the atrial septum and rear right atrial wall in the pacing group increased remarkably, while the CVIB decreased significantly. The tissue pathology changes of cardiomyocyte hypertrophy and fibrosis, induced by the rapid atrial pacing can produce the changes of the myocardial backscatter signals strengths. The changes in the strength of IBS has a direct relation to the content of myocardial collagens and the degree of myocardial fibrosis, in which when fibrosis exists and the collagen deposits increase, the value of AII of IBS escalates more than that of the normal myocardium and the CVIB is reduced. The normal ultrasonic backscatter presents a periodic change in cardiac cycle and agrees with the periodic systolic and diastolic movement of myocardial fiber, reaching the maximum at the end of diastole and the minimum at the end of contraction. CVIB has no relation to heart rate, cardiac preload, or afterload changes, and its value changes due to the changes in myocardial contractive power [14,15].

When rapid AF induces atrial fibrosis, the atrial wall becomes stiff, the adaptability and laxity are reduced, and atrial wall mobility decreases. Therefore, the CVBI will be reduced.

Conclusions

Ultrasonic backscatter technology is reliable in detecting rapid atrial arrhythmia and myocardial fibrosis and it can be used to evaluate the severity of myocardial fibrosis.

References:

1. Zhou ZQ, Hu DY, Chen J et al: [An epidemiological survey of atrial fibrillation in China.] Cliin J lntern Med, 2004; 43(7): 491–94 [in Chinese]

2. Nattel S: New ideas about atrial fibrillation 50 years on. Nature, 2002; 415(2): 219–26

3. Li F, Gan T, Jiang T et al: Expression of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in atrial tissue in the elderly dogs with persistent atrial fibrillation. Journal of Chinese Practical Diagnosis and Therapy, 2012; 10(3): 223–26

4. Ducharme A, Swedberg K, Pfeffer MA et al: Prevetion of atrial fibrillation in patients with symptomatic chronic heart failure by candesartan in the candesartan in heart failure: assessment of reduction in mortality and morbidity (CHARM) program. Am Heart J, 2006; 152: 86–92

5. Xiong R, Zhong G: Atrial fibrosis and atrial fibrillation. Chinese Journal of Cardiac Pacing and Electrophysiology, 2009; 23(5): 450–52

6. Anné W, Willems R, Holeman P et al: Self-terminating AF depends on electrical remodeling while persistent AF depends on additional structural changes in a rapid atrially paced sheep model. J Mol Cell Cardiol, 2007; 43: 148–58

7. Shanglei Y, Qina Z, Hua C et al: Preventive role of atorvastatin in atrial fibrillation and its electrophysiological mechanism. Journal of Chinese Practical Diagnosis and Therapy, 2012; 26(5): 449–51

8. Naili D, Fang J, Ruizhi D: Evaluation of hypertensive myocardial fibrosis by quantitative myocardial acoustic densitometry. Chin J Hypertension, 2006; 14(12): 970–72

9. Cha TJ, Ehrlich JR, Zhang L et al: Dissociation between ionic remodeling and ability to sustain atrial fibrillation during recovery from experimental congestive heart failure. Circulation, 2004; 109: 412–18

10. Anné W, Willems R, Holeman P et al: Self-terminating AF depends on electrical remodeling while persistent AF depends on additional structural changes in a rapid atrially paced sheep model. J Mol Cell Cardiol, 2007; 43(2): 148–58

11. Ke D, Xu C, Zeng G et al: Remodeling of atrial collagens in patients with atrial fibrillation. Medical journal of the Chinese Ppeople's Armed Police Forces, 2008; 9(1): 36–41

12. Chrysostomakis SI, Karalis IK, Simantirakis EN et al: Angiotensin II type I receptor inhibition is associated with reduced tachyarrhythmia-induced ventricular interstitial fibrosis in agoatatrial fibrillation model. Cardiovasc Drugs Ther, 2007; 21(5): 357–65

13. Mohua G, Ruizhi N, Yun G et al: Evaluation of myocardiopathy by ultrasonic integrated backscatter in type 2 diabetes mellitus. Journal of Chinese Modern Imaging, 2005; 2(5): 398–400

14. Ayen M, Popp RL: Influence of the orientation of myocardial fibers on echocardiographic images. Am J Cardiol, 1987; 60: 144–52

15. Brim PD, Brien WD, Rhyne TL et al: Relation of ultrasonic backscatter and acoustic propatation properties to myofibrillar length and myocardial thickness. Circulation, 1995; 91: 171–75