

An Experimental Study on Rapid Localization of the Atrioventricular Node During Open-Heart Surgery

Qihong Huang, MD,¹ Yangyang Li, MD,² Chunhui Xiao, MD,¹ Yingjie Yang, MD,² Minghua Zhu, MD,¹ Guirong Lu, MD,¹ Ke Chen, MD,¹ Shaohui Song, MD,¹ Libo Jin, MM,¹ Hao Wu, MD¹

¹Department of Cardiothoracic Surgery, The Affiliated Hospital of Hangzhou Normal University, Hangzhou City, Zhejiang Province, China; ²Department of Cardiothoracic Surgery, The Third Affiliated Hospital of Xinxiang Medical University, Xinxiang City, Henan Province, China

ABSTRACT

Background: To verify the validity and feasibility of using a mechanical compression method to locate the atrioventricular node in open-heart surgery.

Methods: Ten healthy miniature pigs were used to establish an animal model of the beating heart under cardiopulmonary bypass. During the operation, the atrioventricular node and its surrounding areas were stimulated by mechanical compression (mechanical compression method), and the occurrence of complete atrioventricular block was judged by real-time electrocardiograph monitoring and direct observation of the heart rhythm to identify the position of the atrioventricular node. The final localization of the atrioventricular node was determined using the iodine staining method, and the results were used as the "gold standard" to test the effectiveness and feasibility of the mechanical compression method for locating the atrioventricular node.

Results: With the beating heart model, complete atrioventricular block occurred after mechanical compression of the "atrioventricular node" area in 10 pigs. Nine pigs regained normal conduction immediately after the compression was released, and one pig failed to recover. No atrioventricular block or other arrhythmias occurred after mechanical compression of the "non-atrioventricular node" area. The sensitivity of the method was 86.6%, specificity was 100.0%, misdiagnosis rate was 0.0%, missed diagnosis rate was 13.4%, positive predictive value was 100.0%, negative predictive value was 97.9%, positive likelihood ratios were $+\infty$, negative likelihood ratios were 13.4%, accuracy was 98.1%, and diagnostic odds ratio was $+\infty$.

Conclusion: This study innovatively proposes the application of the mechanical compression method to locate the atrioventricular node during operation and preliminarily proves that this method is effective and feasible through animal experiments.

Received June 3, 2020; received in revised form June 24, 2020; accepted July 6, 2020.

Correspondence: Hao Wu, The Affiliated Hospital of Hangzhou Normal University, No.126 Wenzhou Road, Gongsbu District, Hangzhou City, Zhejiang Province, China 310011; 8613989821921 (e-mail: 13989821921@163.com).

INTRODUCTION

High/complete atrioventricular block (CAVB) is a serious complication of open-heart surgery, and the main cause is accidental injury of the cardiac conduction system [Aziz 2013; Anderson 2012]. The atrioventricular node (AVN) is an important part of the heart conduction system. During repair of congenital heart diseases, CAVB often occurs after operation because of damage to the area. The AVN cannot be identified by the unaided eye; when an operation involves the area of the AVN, the location of the node is mainly determined

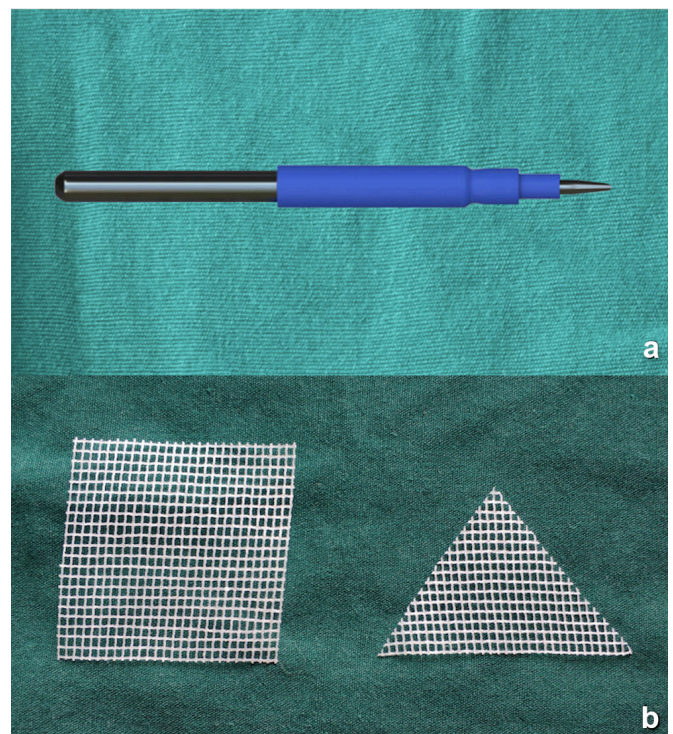


Figure 1. a, The "positioning pen" is made of metal and has a smooth head (compression end). The cross-sectional diameter of the head is approximately 1 mm. b, The "coordinate positioner" is made of poly-ester fibers with moderate softness and hardness. It can be tailored into arbitrary shapes and sizes according to need. The size of each lattice is approximately 1 mm*1 mm.

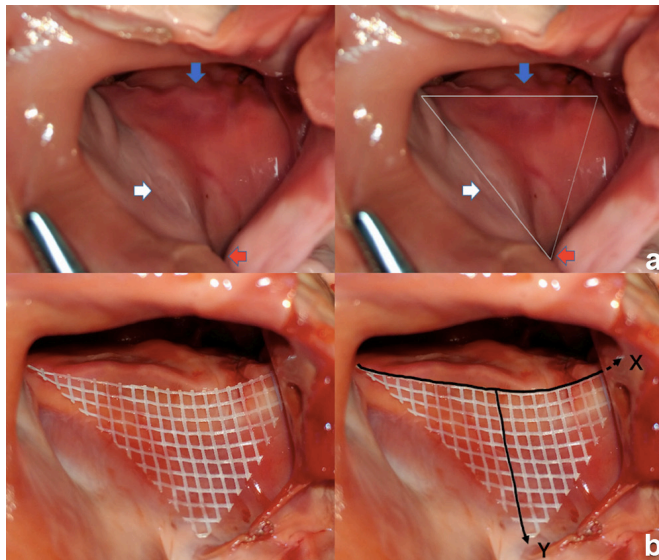


Figure 2. a, The red arrow is the coronary sinus orifice, the blue arrow is the attachment edge of the tricuspid septal valve, and the white arrow is the oval fossa. The three arrows form a “triangular area.” b, The shapes and size of the coordinate positioner are cut into the same triangular area and overlap it on the myocardial tissue of the triangular area. An edge overlapping the attachment edge of the tricuspid septal valve is defined as the “X” axis, and the vertical line of the point is defined as the “Y” axis, to record the position of any point on the coordinate axis.

based on the anatomical location and the experience of the operator. Most open-heart surgeries under cardiopulmonary bypass are performed in the absence of electrical activity during cardiac arrest; thus, once the AVN is injured during the operation, it will not be found in time. If there are anatomical variations in the AVN or if the corresponding location of the pathological anatomy is complex, accurate location of the AVN is increasingly difficult [Azab 2013], and the probability that the AVN will be injured greatly is increased. Therefore, we designed a series of experiments to solve this problem.

MATERIALS AND METHODS

The experimental protocol was approved by the Animal Ethical and Welfare Committee of Zhejiang Chinese Medical University and all animals received humane care.

Experimental materials: Ten miniature pigs weighing 30–40 kg were purchased from Shanghai Jiagan Biological Technology Co., Ltd. and were randomly numbered P1–P10. Materials included Lugol's iodine solution (5%) (Fujian Weizhenyuan Pharmaceutical Co., Ltd), HL20 cardiopulmonary bypass machine (Maquet Company of Germany), children's membrane oxygenator (Kuwait Medical Equipment Co., Ltd., of Dongguan), AS-01-0007 animal anesthesia machine (Guanguan Da Technology Development Co., Ltd., of Beijing), and ACT Plus automatic coagulation time meter (Medtronic Company of

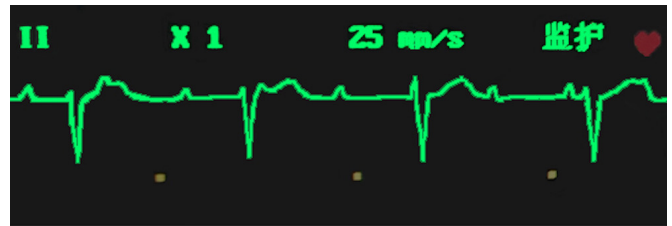


Figure 3. The ECG waveform of CAVB was observed on the ECG monitor after mechanical compression.

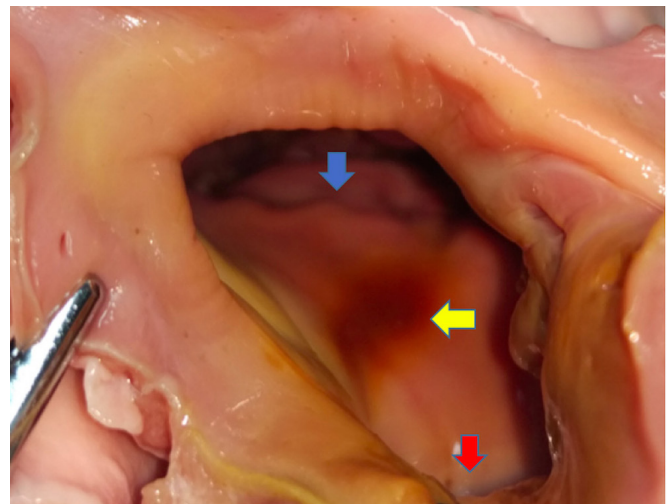


Figure 4. The yellow arrow indicates the myocardium stained with Lugol's iodine solution appears brown-black.

America). The homemade mechanical compression “positioning pen” figure 1a, and “coordinate positioner” figure 1b, are depicted below figures 1a and 1b.

Animal anesthesia administration and cardiopulmonary bypass management: The experimental animals underwent preoperative fasting and abstinence for 12 hours. Atropine (0.02 mg/kg), sumianxin (0.1 ml/kg) and 3% pentobarbital sodium (3 mg/kg) were injected intramuscularly for basic anesthesia. The auricular vein was punctured for infusion, and the femoral artery was used for continuous arterial blood pressure monitoring. A single-lumen endotracheal tube with a diameter of 6.0–6.5 mm was inserted into the trachea, and the depth of intubation was 26–28 cm. The anesthesia machine controlled the breathing with a tidal volume of 10 ml/kg, breathing frequency of 18–20 times/min, breathing ratio of 1:2, and inhaled oxygen concentration of 100%. Intravenous propofol (5 mg/kg/h), vecuronium (0.2 mg/kg/h), and continuous inhalation of 2–5% isoflurane were used to maintain anesthesia. The blood was heparinized with 3 mg/kg heparin and then cooled to 32–34°C. The intraoperative perfusion flow was 80–120 ml/min/kg, the mean arterial pressure (MAP) was 60–80 mmHg, and the activated coagulation time (ACT) was more than 750 s.

Establishment of a model of beating heart surgery under cardiopulmonary bypass: After median thoracotomy, aortic

Table 1. Four cases of mechanical compression localization

Location results of mechanical compression	Gold standard		Total
	AVN* spot	Non-AVN† spot	
Positive point	Numbers of true positive	Numbers of false positive	N1
Negative point	Numbers of false negative	Numbers of true negative	N2
Total	N3	N4	

*atrioventricular node; †non-atrioventricular node

intubation was performed at the distal end of the ascending aorta close to the innominate artery, the superior vena cava was freed from the pericardium, right pleura was opened, inferior vena cava was freed from the pericardium, and superior and inferior venae cavae were directly intubated. A left ventricular drainage tube was inserted at the junction of the right superior pulmonary vein and left atrium. The superior and inferior venae cavae were blocked, although the aorta was not blocked; cardioplegia was not perfused, and sinus rhythm was maintained.

Location of the AVN by mechanical compression: The right atrium was incised under a beating empty heart, and the coordinate positioner was overlapped on the myocardial tissue of the "triangular area" figure 2a, according to research reports [Guo 2001], the anatomical position of the AVN of the pig heart is located at the front of the coronary sinus orifice, above the tricuspid septal cusp, and in the anterior and inferior part of the oval fossa. The myocardial tissue in the triangle area was stimulated by mechanical compression with a "positioning pen" along the small grid of the "coordinate positioner," the pressure increased gradually but could not exceed the visible rupture of endocardial, and the compression time was no more than 10 seconds. Changes in the cardiac rhythm or electrocardiograph (ECG) on the monitor were observed when pressure was applied. Once CAVB occurred, the compression was terminated, and the "point" was recorded as a positive point. Compression of the next position after the ECG was restored to normal. If CAVB did not occur on compression throughout, the "point" was recorded as a negative point. The number of positive (N1) and negative (N2) points and the corresponding coordinates of each point were recorded in each experimental pig figure 2b.

Location of the AVN by iodine staining: In the same experimental pig, after mechanical compression was completed, the right atrium was washed with normal saline. The blood was washed away, and residual moisture of the endocardium was dried by gauze. The 5% Lugol's iodine solution was evenly applied to the endocardium of all parts of the right atrium with a cotton ball, and the color of the myocardium was observed after one minute. At the same time, the coordinate positioner was again placed in the "triangular area," and the coloring "point" in the area was recorded as an AVN spot. The non-coloring "point" in the area was recorded as a non-AVN spot. The number of AVN (N3) and non-AVN (N4)

spots and the corresponding coordinate positions of each point were recorded using the coordinate positioner.

Statistical analysis: According to the results of mechanical compression localization and the "gold standard" (iodine staining results), the numbers of true positive, false positive, false negative, and true negative were obtained in each pig (Table 1).

The sensitivity, specificity, misdiagnosis rate, missed diagnosis rate, positive predictive value, negative predictive value, positive likelihood ratios, negative likelihood ratios, accuracy, and diagnostic odds ratio of the mechanical compression method for locating the AVN were calculated, based on the results of iodine staining.

RESULTS

Compression localization results: Nine of the 10 experimental pigs were observed for compression of myocardial tissue in the triangle area. The beating rhythm of the heart significantly slowed, and the ECG waveform of CAVB appeared on the ECG monitor (Figure 3). One pig (P4) had no atrioventricular block or other arrhythmias after repeated compression in the triangular area. CAVB occurred in the heart, during mechanical compression above the anterior part of the oval fossa. Of all the experimental pigs that had CAVB after compression, nine recovered to normal after decompression. The remaining pig (P7), which was compressed above the tricuspid septal cusp, had persistent CAVB after decompression even though atropine and isoproterenol intravenously were given for 10 minutes.

Staining localization results: In all 10 experimental pigs, the myocardial tissue was clearly stained, and the myocardial tissue in the chromogenic area was brown-black; the remaining myocardium was not stained (Figure 4). The stained cardiac tissues in nine of the experimental pigs were located in the "triangular area," which was close to the tricuspid septal cusp, and all the positive points were located in the area of the AVN spot. The stained tissue in one pig (P4) was located above the anterior part of the oval fossa.

Statistics: As shown in Table 2, we successfully located the atrioventricular nodes in all pigs except one (P7) in which irreversible CAVB occurred (Table 2). In this table, true positive means the "point" at which CAVB occurred on compression and could be stained with iodine solution; false positive

Table 2. Compression and staining localization results

Result	Experimental pig serial number									
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Numbers of true positive	13	16	15	0	14	17	-	16	15	17
Numbers of false positive	0	0	0	0	0	0	-	0	0	0
Numbers of false negative	3	3	2	0	2	3	-	1	3	2
Numbers of true negative	95	98	94	113	96	91	-	95	93	97

Table 3. Different positioning methods of atrioventricular node

Methods	Advantages	Disadvantages
Compression localization	1. Easy to operate 2. High accuracy	Improper operation may cause damage to the cardiac conduction system
Staining localization	High accuracy	Often causes damage to endocardium
Anatomic localization	Simple and fast	1. Rough positioning 2. If there are anatomical variations in the AVN, it can't effectively be identified
Electrophysiological delineation	None	1. Complicated 2. Only the His bundle and the proximal bundle branch can be located 3. Professional knowledge of cardiac electrophysiology is required

means the "point" at which CAVB occurred on compression but could not be stained with iodine solution; true negative means the "point" at which CAVB did not occur on compression and could not be stained with iodine solution; false negative means the "point" at which CAVB did not occur on compression but could be stained with iodine solution.

The analysis of the mechanical compression method revealed a sensitivity of 86.6%, specificity of 100.0%, misdiagnosis rate of 0.0%, missed diagnosis rate of 13.4%, positive predictive value of 100.0%, negative predictive value of 97.9%, positive likelihood ratios of $+\infty$, negative likelihood ratios of 13.4%, accuracy of 98.1%, and diagnostic odds ratio of $+\infty$.

DISCUSSION

In this study, we found that CAVB occurs in the heart after AVN is stimulated by mechanical compression. If the compression stimulus is relieved within a certain period of time, the heart can resume normal conduction. Accordingly, we invented the "mechanical compression method" and successfully applied it to locate the AVN during surgery.

In our experiment, we observed that CAVB appeared in the heart after we applied moderate mechanical pressure stimulation to the AVN of the pig, and the heart returned to normal conduction immediately after the stimulus was relieved. This phenomenon indicates that there is "dysfunction" after the AVN is stimulated by compression and that this "dysfunction" is reversible to a certain extent. The mechanism of this phenomenon is not clear but may be related to the change in the membrane potential of myocardial cells [Zeng 2018]. Normally, myocardial cells need to undergo three stages to

produce a single excitation: an effective refractory period, relative refractory period, and extraordinary period. The membrane potential levels vary in different stages. During the relative refractory period, the impulse (threshold stimulation) from the sinoatrial node cannot excite the myocardial cells. However, if a suprathreshold stimulation is given at this time, the myocardial cells in this area can become excited and then enter the effective refractory period. If AVN myocardial cells receive continuous suprathreshold stimulation within a certain period of time, the myocardial cells in this area will enter the effective refractory period directly after the relative refractory period but not into the extraordinary period; as a result, the impulse is released by the sinoatrial node and is not transmitted to the ventricle all the time. After the suprathreshold stimulation disappears, the excitability of myocardial cells gradually returns to normal, and the impulse from the sinoatrial node could be normally transmitted downward. The mechanical compression stimulus of the "positioning pen" on the AVN is equivalent to the suprathreshold stimulus, which could lead to reversible CAVB. In their research study, Del et al. found that the electrical signals between myocardial cells mainly are transmitted through the gap junction, which is a membrane channel structure between adjacent cells, and its opening and closing can be affected by membrane potential [Del 2015]. Mechanical compression stimulation may induce the closure of the gap junction between cardiac myocytes by affecting the membrane potential level, leading to the occurrence of CAVB.

The cardiac conduction system is composed of specialized myocardial cells, which contain a substantial amount of glycogen and can react with iodine [Yuan 2015]. Lugol's iodine solution is composed of iodine and potassium iodide in a certain proportion and can effectively be used for in vivo

staining of the cardiac conduction system [Allen 1959; Wei 2010]. In our experiment, mechanical compression stimulation was applied to a specific part of the right atrium of the pigs. The typical electrocardiographic manifestations of CAVB were observed in the ECG monitors of all subjects. In addition, the heartbeat was slowed under direct visualization, showing that mechanical compression stimulation could indeed affect the conduction function of the AVN. To confirm whether the location where the myocardial tissue is in contact when the mechanical compression method produces an "action" is the area of the AVN that we wish to locate, we used Lugol's iodine staining to determine the exact location of the AVN. The experimental results show that the specificity of the mechanical compression method is 100.0%, and the misdiagnosis rate is 0.0%. Moreover, these results show that as long as CAVB occurs in the heart during mechanical compression stimulation, we can consider the compression area to be the location of the AVN. When we need to operate in the right atrium and are not sure whether the AVN passes through the area, we can apply mechanical compression to that area. If CAVB occurs after compression, the area likely is to be the location of the AVN, and we need to avoid any injuries to that area. Although the sensitivity of the "mechanical compression method" is 86.6%, the missed diagnosis rate still is 13.4%, which shows that the method cannot completely identify some areas of the AV node. In our experiment, we also determined the "gold standard" as the AV node area and the "mechanical compression method" as the non-AV node area of myocardial tissue ligation. We found that CAVB did appear in the heart, so we speculated that the reason the sensitivity could not reach 100.0% may be due to the relative insufficiency of the compressive force caused by atrioventricular nodal tissues located far below the endocardial surface.

In this study, the AVN was not found in the "triangular area" of one of the experimental pigs (P4) but was located above the anterior part of the oval fossa during the mechanical compression process. We speculated that the location of the AVN was variable, which was confirmed by iodine staining. In addition, one of the experimental pigs (P7) showed persistent CAVB after decompression. After intravenous atropine and isoproterenol, the sinus rhythm was not restored, and irreversible damage to the AVN was considered to have occurred. The reason may be that we did not smoothly carry out the positioning process and unconsciously increased the pressure, prolonging the compression time. According to Tian and Ma, continuous myocardial ischemia can lead to the closure of gap junctions between cells (i.e., electrical decoupling), when the conduction velocity slows down or the conduction direction is disordered [Tian 2016]. Therefore, we believe that a "positioning pen" that is equipped with pressure sensors and timers should be designed in order to determine a safe range for accepting compressive forces and a range of compression time for the AVN, and the anterior segment of the "positioning pen" can bend arbitrarily to better adapt to the location of the AVN under various clinical pathological and anatomical conditions.

This study explored the method of intraoperative localization of the AVN in pigs and preliminarily proved the effectiveness of

the "mechanical compression method." As we can see in Table 3, this method has certain advantages over other clinical positioning methods (Table 3). However, only 10 samples were used in the experiment, which may have impacted the accuracy of the experimental results. To solve this problem, we will carry out a study using a larger sample size in future experiments. Because of the limited conditions, the animals were sacrificed at the end of the experiment, and the function of the AVN was not observed further. In addition, further improvements are needed in designing the positioning pen. We need to overcome these challenges in our future study. Moreover, this experiment is based on animal experiments carried out in pigs. Although pig disease characteristics, tissue structure, physiological metabolism and cardiovascular system anatomy are similar to those of humans [Hu 2014], there are differences between the two species. Whether the mechanical compression method is effective in locating the AVN in the human heart needs to be confirmed by clinical trials.

CONCLUSIONS

This study innovatively proposes the application of the mechanical compression method to locate the AVN during surgery and preliminarily proves that this method is effective and feasible through animal experiments. The findings of this study provide a new idea for rapidly locating the AVN during open-heart surgeries and lay a foundation for developing a more secure positioning pen to carry out clinical trials.

ACKNOWLEDGEMENTS

Funding details: This work was supported by the Zhejiang Province Public Welfare Technology Application Research Project under Grant number LGF18H020002.

REFERENCES

- Allen P, Lederman JJ, Pearl GJ. 1959. Prevention of surgical heart block by the use of supravital stain. *Journal of Thoracic & Cardiovascular Surgery*. 38(1):57-61.
- Anderson JB, Czosek RJ, Knilans TK, Meganathan K, Heaton P. 2012. Postoperative Heart Block in Children with Common Forms of Congenital Heart Disease: Results from the KID Database. *Journal of Cardiovascular Electrophysiology*. 23(12):1349-1354.
- Azab S, El-Shahawy H, Samy A, Mahdy W. 2013. Permanent complete heart block following surgical closure of isolated ventricular septal defect. *Egyptian Journal of Chest Diseases & Tuberculosis*. 62(3):529-533.
- Aziz PF, Serwer GA, Bradley DJ, et al. 2013. Pattern of recovery for transient complete heart block after open heart surgery for congenital heart disease: duration alone predicts risk of late complete heart block. *Pediatric Cardiology*. 34(4):999-1005.
- Del RS, Moscato S, Bianchi F, et al. 2015. Altered expression of connexin 43 and related molecular partners in a pig model of left ventricular dysfunction with and without dipyridamole therapy. *Pharmacological Research*. 95-96:92-101.

Guo ZK, Xu ZP, Zhao BQ. 2001. Histological observation and quantitative analysis of atrioventricular node in pigs. *Chinese Journal of Anatomy* 24 (2): 153-157.

Hu YY, Lin H, Huang AL, et al. 2014. Establishment of mild hypothermic cardiopulmonary bypass on beating heart for Guangxi bama miniature pig. *Chinese Journal of New Clinical Medicine*. 7 (2): 103-105.

Tian P, Ma H. 2016. Research progress of connexin 43 and arrhythmia. *Chinese Journal of Evidence-based Cardiovascular Medicine*. 8 (2): 238-239.

Wei ZW, Ren P. 2010. Comparison of two methods for displaying cardiac conduction system. *Journal of Liaoning University of Traditional Chinese Medicine*. (1): 198-199.

Yuan XL, Xu F, Li YQ, et al. 2015. Anatomical observation of Purkinje fiber dense areas in bovine and canine ventricles. *Chinese Journal of Anatomy*. 38 (2): 215-217.

Zeng XR. 2018. The electrophysiology of the heart and physiological characteristics. In: Zhu DN, Wang TH, editors. *Physiology*. 8nd en. Beijing: People's Health Publishing House, p103-104.