Int J Clin Exp Med 2016;9(7):12751-12756
www.ijcem.com /ISSN:1940-5901/IJCEM0023276

Original Article

Impact of *shenxian shengmai* oral liquid on Ca^{2+}-Mg^{2+}-ATPase and myocardial Cx43 expression in rats with chronic arrhythmia

Wenqi Yang[1], Zhaolong Xu[1], Shudan Mao[2]

Departments of [1]Cardiology, [2]Hematology, The First Affiliated Hospital of Liaoning Medical University, Jinzhou, China

Received January 5, 2016; Accepted April 1, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Chronic arrhythmia is common in clinic. *Shenxian shengma*i oral liquid shows definite curative effect on chronic arrhythmia treatment through improving sinoatrial node conduction and increasing heart rate. This study established chronic arrhythmia rat model using propranolol to observe *shenxian shengmai* oral liquid effect on Ca^{2+}-Mg^{2+}-ATPase and myocardial Cx43 expression in rats with chronic arrhythmia, and further discussed its molecular mechanism of treating chronic arrhythmia. Male SD rats, weighted 200-220 g, were randomly divided into model group (A), drug group (B), and control group (C) with 15 in each group. Propranolol was adopted to establish the chronic arrhythmia model *in vivo*. The rats in group B received *shenxian shengmai* oral liquid, while the rats in group C and A received equal volume of saline for continuous 14 days. The heart rate was observed, Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase level in myocardium were detected by spectrophotometric method, and myocardial Cx43 and Kir2.1 protein expression was determined by Western blot. The rats in group A showed significantly lower heart rate than that in group C, together with prolonged P-R interval and QRS duration. While Na^{+}-Mg^{2+}-ATPase, Ca^{2+}-Mg^{2+}-ATPase, Cx43, and Kir2.1 level decreased. Compared with group A, the rats in group B presented obviously higher heart rate, shorter P-R interval and QRS duration, and elevated Na^{+}-K^{+}-ATPase, Ca^{2+}-Mg^{2+}-ATPase, Cx43, and Kir2.1 protein expression. In conclusion, our studies showed that *Shenxian shengmai* oral liquid treatment on chronic arrhythmia is related to Na^{+}-Mg^{2+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase activity elevation and increase of Cx43 and Kir2.1 protein.

Keywords: *Shenxian shengmai* oral liquid, arrhythmia, Ca^{2+}-Mg^{2+}-ATPase, Cx43

Introduction

Chronic arrhythmia is a clinic familiar disease, common in sinoatrial block, sinus bradycardia, atrioventricular block, and sick sinus syndrome [1, 2]. Currently, the effective method of treating chronic arrhythmia in clinic is electronic pacemaker. However, there exists some problems in electronic pacemaker implantation, such as electrode position instability, infection, lack of automatic response to neurotransmitter changes, limited battery life, and electromagnetic interference, etc [3, 4]. For special patients, especially children, growth development of let the previously installed pacemaker cannot meet the needs [5, 6], thus limited the pacemaker application in special patients. Improper western medicine used in clinic may induce arrhythmia. *Shenxian shengmai* oral liquid is adopted for chronic arrhythmia with confirmed curative effect and fewer side effects. It shows good curative effect in improving the clinical symptoms, electrocardiogram, and heart rate, which can be used for long-term in the patients not suitable for cardiac pacemaker installation. At present, the mechanism of *shenxian shengmai* oral liquid has not been fully elucidated. Intracellular calcium overload is an important reason to induce arrhythmia, while Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase play important roles in maintaining intracellular calcium homeostasis. Their activities declined in the myocardium of chronic arrhythmia patients [7, 8]. The main material basis of myocardial potential opening is the gap junction in the cells. It plays an important role in electric cou-

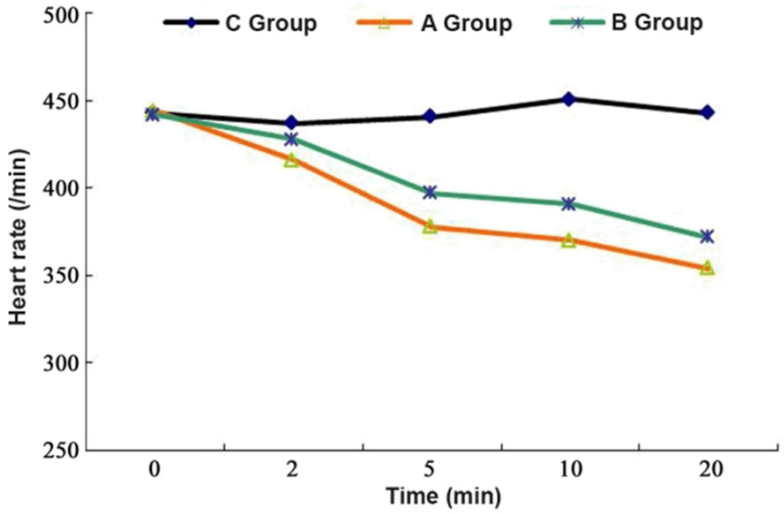

Figure 1. The impact of shenxian shengmai oral liquid on heart rate in rats with chronic arrhythmia. Group A. Model group; B. Shenxian shengmai oral liquid group; C. Control. $^{\Delta}P < 0.05$, compared with group C; $^{*}P < 0.05$, compared with group A.

pling between cells and impulse conduction that mainly relies on gap junction protein changes to regulate gap junction function [9, 10]. The main gap junction protein expressed on ventricular muscle cells is Cx43. Under pathological condition, humoral factors level is abnormal, impacting gap junction protein expression, transfer and distribution. It further changes gap junction structure and function change and disrupts the communication function between myocardial cells, resulting in the probability of myocardial potential loss of coupling elevation and inducing arrhythmia [11, 12]. This study established chronic arrhythmia rat model using propranolol to observe the impact of *shenxian shengmai* oral liquid on Na^{+}-K^{+}-ATPase, Ca^{2+}-Mg^{2+}-ATPase, Cx43, and Kir2.1 expression in rats with chronic arrhythmia, and further discussed its molecular mechanism of treating chronic arrhythmia to provide basis for clinical treatment.

Materials and methods

Experimental animals and grouping

Healthy male SD rats in eight-week old and weighted 200-220 g were provided by Liaoning Medical University, animal experiment center (license key: SYXK-2013-0025). The rats were raised in SPF grade laboratory, and their diet conformed to the standard. The rats were randomly divided into model group (A), drug group (B), and control group (C) with 15 in each group. Propranolol was adopted to establish the chronic arrhythmia model *in vivo*. The rats in group B received *shenxian shengmai* oral liquid (4.2 ml/kg), while the rats in group C and A received equal volume of saline for continuous 14 days. Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of the First Affiliated Hospital of Liaoning Medical University.

Investigational product and reagents

Shenxian shengmai oral liquid was bought from Shandong Buchang pharmaceutical co. LTD (Shandong, China). Rabbit anti-rat Cx43 and Kir2.1 monoclonal antibodies were provided by Boster (Wuhan, China). Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase detection kits were supplied by Nanjing Jiancheng biological engineering (Nanjing, China).

Animal model preparation

Chronic arrhythmia rat model was established according to the reference [13]. Propranolol is a type of β-blocker that can block adenylate cyclase activation, reduce calcium channel open, and slow down heart rate. Urethane (30%, 1.2 g/kg) was intraperitoneal injected at 2 hours after the last delivery. The rat was fixed at supine position and the electrocardiogram II lead was recorded. The rats with abnormal electrocardiogram were ruled out. Propranolol was intraperitoneal injected at 5 mg/kg, and the electrocardiogram was recorded at 0, 2, 5, 10, and 20 min after injection. The rats were euthanized and the heart tissue was taken for further detection.

Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase activity detection

Total protein was extracted from the right atrium (sinoatrial node) myocardial tissue and quantified by Coomassie brilliant blue. Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities were detected according to the manual.

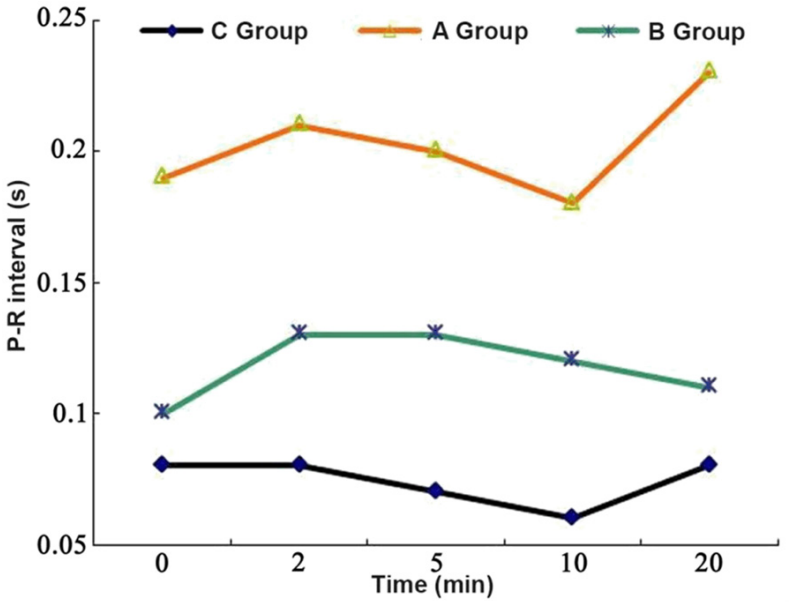

Figure 2. The impact of shenxian shengmai oral liquid on P-R interval in rats with chronic arrhythmia. Group A. Model group; B. Shenxian shengmai oral liquid group; C. Control. $^{\Delta}P < 0.05$, compared with group C; $^{*}P < 0.05$, compared with group A.

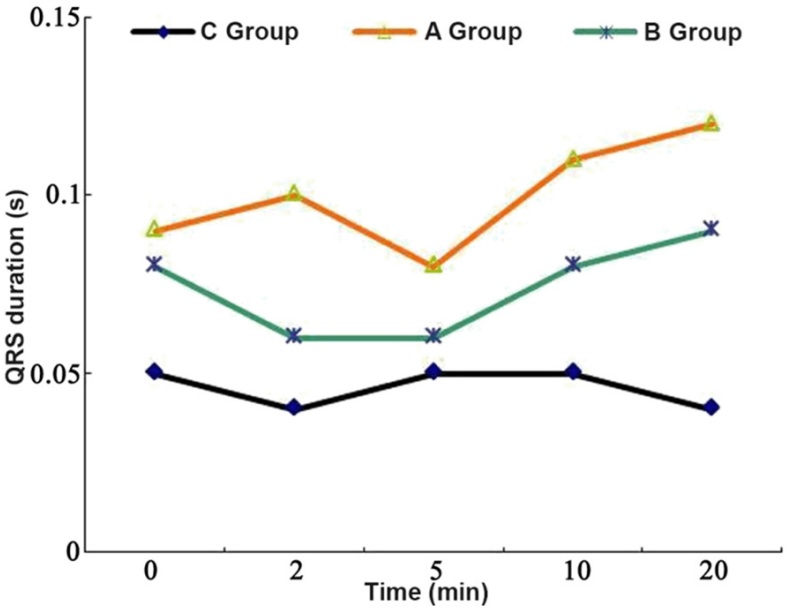

Figure 3. The impact of shenxian shengmai oral liquid on QRS duration in rats with chronic arrhythmia. Group A. Model group; B. Shenxian shengmai oral liquid group; C. Control. $^{\Delta}P < 0.05$, compared with group C; $^{*}P < 0.05$, compared with group A.

Western blot

Total protein was separated by SDS-PAGE and transferred to PVDF membrane. After blocked by skim milk, the membrane was incubated with Cx43 (1:1000), Kir2.1 (1:500), and GAPDH (1:1000) primary antibodies at 4°C over night. Then the membrane was incubated with secondary antibody at 1:2000 for 1 h and washed by TBST. At last, the membrane was treated with enhanced chemiluminescent for visualization of positive bands.

Statistical analysis

All the statistical analysis was performed by SPSS 19.0 software. The data was presented as mean ± SD. One-way ANOVA and LSD test were used for comparison in different groups. $P < 0.05$ was considered as statistically significant.

Results

Impact of shenxian shengmai oral liquid on heart rate

Compared with control group, the descend range of rats' heart rate in model group increased significantly in each time point ($P < 0.05$), indicating that propranolol intraperitoneal injection successfully established chronic arrhythmia model. The descend range in *shenxian shengmai* oral liquid group decreased obviously compared with model group ($P < 0.05$) (**Figure 1**).

Impact of shenxian shengmai oral liquid on P-R interval

Compared with that in control group, the P-R interval was prolonged in rats in model group ($P < 0.05$). While compared with model group, P-R interval was markedly shortened in drug group

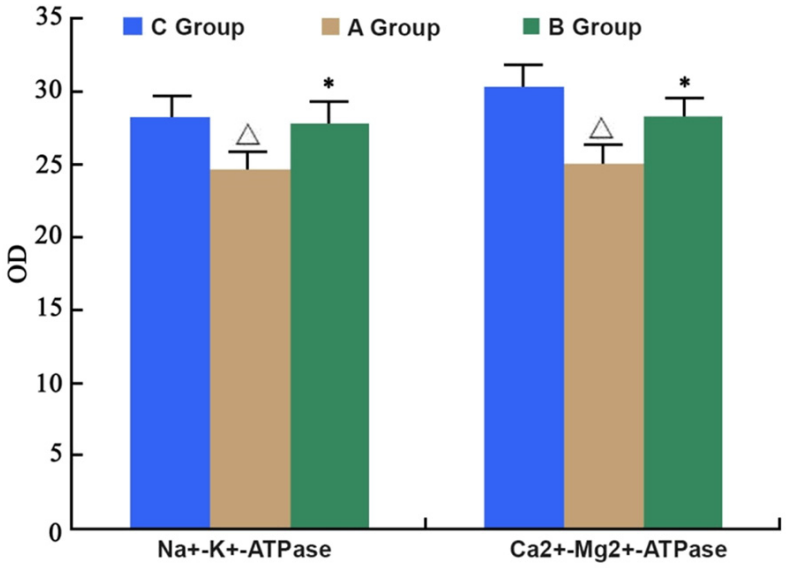

Figure 4. The impact of shenxian shengmai oral liquid on Na^+-Mg^{2+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase levels in myocardium. Group A. Model group; B. shenxian shengmai oral liquid group; C. Control. $^{\Delta}P < 0.05$, compared with group C; $^{*}P < 0.05$, compared with group A.

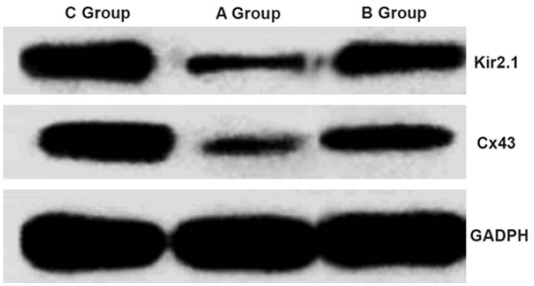

Figure 5. The impact of shenxian shengmai oral liquid on Cx43 and Kir2.1 protein expression in myocardium. Group A. Model group; B. Shenxian shengmai oral liquid group; C. Control.

in each time point ($P < 0.05$), suggesting that *shenxian shengmai* oral liquid can improve P-R interval in chronic arrhythmia (**Figure 2**).

Impact of shenxian shengmai oral liquid on QRS duration

QRS duration was prolonged in rats in model group compared with control group ($P < 0.05$), whereas it was shortened obviously in drug group in each time point compared with that in the model group ($P < 0.05$), revealing that *shenxian shengmai* oral liquid can improve QRS duration in chronic arrhythmia (**Figure 3**).

Impact of shenxian shengmai oral liquid on Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase levels in myocardium

Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities decreased in rats in model group compared with control group ($P < 0.05$), but increased significantly in drug group in each time point compared with those in the model group ($P < 0.05$) (**Figure 4**).

Impact of shenxian shengmai oral liquid on Cx43 and Kir2.1 protein expression in myocardium

Cx43 and Kir2.1 protein expression was reduced in rats in model group compared with control group but elevated obviously in drug group (**Figure 5**).

Discussion

The pathogenesis of chronic arrhythmia has not been fully elucidated. Studies have shown that it is related to conductive fiber and autoautorhythmic cell degenerative disease, ion channels abnormal, vagus nerve tension abnormal, and myocardial cells ion transport [14, 15]. As a common ionic pump, Na^+-K^+-ATPase is widespread on cell membrane. Its main function is transmembrane transporting Na^+ and K^+. At the same time of ATP hydrolysis, it can confront electrochemical gradient on each side of the cell membrane, and maintain the concentration difference of Na^+ and K^+. The main function of Ca^{2+}-Mg^{2+}-ATP is to active transport Ca^{2+} across the cell membrane to extracellular and absorb Ca^{2+} from cytoplasm to sarcoplasmic reticulum. Ca^{2+} release disorder in the calcium pool may cause sarcoplasmic reticulum permeability decline for Ca^{2+}, leading to decreased calcium influx, slow down the pacemaker cell automatic depolarization in sinoatrial node, and induce chronic arrhythmia. Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase both involved in myocardial cell membrane ion channel and ion trans-

port [16, 17]. Ion transport between myocardial cell membrane is the foundation of action potential formation. Cell membrane permeability is related to ion channel, thus abnormal cardiac ion channel is the main reason for the chronic arrhythmia formation [18, 19]. Our results showed that *shenxian shengmai* oral liquid can elevate Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in the myocardial tissue of chronic arrhythmia rats. It further can keep the normal ion concentration gradient and electrochemical gradient to maintain normal cell energy metabolism. Propranolol can block β receptor in sinoatrial node cells, reduce the myocardial cell autorhythmicity, and slow down heart rate. Shenxian shengmai oral liquid can obviously inhibit heart rate reduction, shorten P-R interval and QRS duration, delay atrioventricular block, and shorten the recovery time caused by propranolol, further confirming its role in treating chronic arrhythmia in clinic.

Gap junction protein in the tissues mainly contains Cx40, Cx43, and Cx45, of which Cx43 is the main connexin between myocardial cells. Cx43 is mainly encoded by GJA1 gene on chromosome 6 and distributes in cluster in myocardial cells. It maintains communication, energy exchange, and chemical signaling between cells. Cx43 protein reduction can cause cell electric coupling loss, and prolong action potential duration and repolarization process, resulting in conduction block. Cx43 plays an important role in maintaining normal development of heart, electrical activity synchronization, and coordination secretion [19, 20]. Abnormal Cx43 expression and distribution reduce electric conduction coupling and intracellular conduction, thus inducing reentry arrhythmia. Homozygous mice with Cx43 gene knockout has normal cardiac structure and systolic function, but dies of spontaneous ventricular arrhythmia in two months. Gene therapy to increase Cx43 expression can play a protective role in fatal arrhythmia [21, 22]. Gap junction structure and function have influential role in arrhythmia occurrence and development. Kir2.1 protein is mainly encoded by $KCNJ_2$ gene on chromosome 17. Kir2.1 protein mediated IK1 can maintain the stability of resting membrane potential. Kir2.1 protein hypofunction or lower expression can reduce the current strength in stage 3 fast repolarization, extend QT interval and action potential duration, and produce arrhythmia [22, 23]. This study revealed that Cx43 and Kir2.1 protein expression level decreased in myocardial tissue of rats in model group, while their level elevated obviously in drug group. It might be one of the mechanisms of shenxian shengmai oral liquid in treating chronic arrhythmia. Cx43 and Kir2.1 protein up-regulation is associated with target genes CJA1 and $KCNJ_2$ expression. Open and close of gap junction channel is closed related to many factors, such as intracellular Ca^{2+} level, intracellular pH, cross model voltage, and gap protein phosphorylation status, etc. Intracellular Ca^{2+} level reduction can decrease the electric conduction in gap junction with dose-dependent. *Shengxian shengmai* oral liquid plays an anti-arrhythmia effect through increasing Ca^{2+}-Mg^{2+}-ATPase and Cx43 expression.

In conclusion, the mechanism of *shenxian shengmai* oral liquid in treating chronic arrhythmia is associated with increased Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activity and Cx43 and Kir2.1 protein expression. *Shenxian shengmai* oral liquid can upregulate the gap junctions or ion channel gene and protein expression, restore intercellular gap junction protein function and IK1 current, and correct action potential, P-R interval, QRS duration, and intercellular conduction velocity to inhibit arrhythmia. Its exact mechanism on regulating Cx43 and Kir2.1 protein expression may be related to GJA1 and $KCNJ_2$ gene, and this needs further investigation.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shudan Mao, Department of Hematology, The First Affiliated Hospital of Liaoning Medical University, Jinzhou 121000, China. Tel: +86-416-4197061; Fax:+86-416-4197061; E-mail: shudan990@163.com

References

[1] Kant S, Holthofer B, Magin TM, Krusche CA, Leube RE. Desmoglein 2-dependent arrhythmogenic cardiomyopathy is caused by a loss of adhesive function. Circ Cardiovasc Genet 2015; 8: 553-563.

[2] McArthur L, Chilton L, Smith GL, Nicklin SA. Electrical consequences of cardiac myocyte: fibroblast coupling. Biochem Soc Trans 2015; 43: 513-518.

[3] Quang KL, Maguy A, Qi XY, Naud P, Xiong F, Tadevosyan A, Shi YF, Chartier D, Tardif JC, Dobrev D, Nattel S. Loss of cardiomyocyte integrin-linked kinase produces an arrhythmogenic cardiomyopathy in mice. Circ Arrhythm Electrophysiol 2015; 8: 921-932.
[4] Su F, Zhao L, Zhang S, Wang J, Chen N, Gong Q, Tang J, Wang H, Yao J, Wang Q, Zhong M, Yan J. Cardioprotection by PI3K-mediated signaling is required for anti-arrhythmia and myocardial repair in response to ischemic preconditioning in infarcted pig hearts. Lab Invest 2015; 95: 860-871.
[5] Sun B, Jiang JF, Zhao CM, Hu CH. Effects of antiarrhythmic peptide 10 on acute ventricular arrhythmia. Asian Pac J Trop Med 2015; 8: 229-233.
[6] Schultz JG, Andersen S, Andersen A, Erik Nielsen-Kudsk J, Nielsen JM. Evaluation of cardiac electrophysiological properties in an experimental model of right ventricular hypertrophy and failure. Cardiol Young 2016; 26: 451-8.
[7] Aiba T, Noda T, Hidaka I, Inagaki M, Katare RG, Ando M, Sunagawa K, Sato T, Sugimachi M. Acetylcholine suppresses ventricular arrhythmias and improves conduction and connexin-43 properties during myocardial ischemia in isolated rabbit hearts. J Cardiovasc Electrophysiol 2015; 26: 678-685.
[8] Ni M, Ruan L, Zhang C. Antiarrhythmic peptide AAP10 prevents arrhythmias induced by protein kinase C activation in rabbit left ventricular wedges. Int Heart J 2015; 56: 234-238.
[9] Rouhollahi E, Zorofchian Moghadamtousi S, Paydar M, Fadaeinasab M, Zahedifard M, Hajrezaie M, Ahmed Hamdi OA, Looi CY, Abdulla MA, Awang K, Mohamed Z. Inhibitory effect of Curcuma purpurascens BI. rhizome on HT-29 colon cancer cells through mitochondrial-dependent apoptosis pathway. BMC Complement Altern Med 2015; 15: 15.
[10] Hu YF, Chen YJ, Lin YJ, Chen SA. Inflammation and the pathogenesis of atrial fibrillation. Nat Rev Cardiol 2015; 12: 230-243.
[11] Kohl P. Structural and functional recoupling of atrial and ventricular myocardium: new conduits for electrical flow. J Am Coll Cardiol 2014; 64: 2586-2588.
[12] Rampazzo A, Calore M, van Hengel J, van Roy F. Intercalated discs and arrhythmogenic cardiomyopathy. Circ Cardiovasc Genet 2014; 7: 930-940.
[13] Shen YJ, Deng WL. Pharmacology. Beijing: People's Medical Publishing House; 2000.
[14] Seki A, Nishii K, Hagiwara N. Gap junctional regulation of pressure, fluid force, and electrical fields in the epigenetics of cardiac morphogenesis and remodeling. Life Sci 2015; 129: 27-34.
[15] Wang M, Shan J, Yang Q, Ma X, Jin S, Guo X, You Q, Tang Y. Antiarrhythmic efficacy of CPUY102122, a multiple ion channel blocker, on rabbits with ischemia/reperfusion injury. Pharmacol Rep 2014; 66: 1022-1030.
[16] Santos-Almeida FM, Girao H, da Silva CA, Salgado HC, Fazan R Jr. Cholinergic stimulation with pyridostigmine protects myocardial infarcted rats against ischemic-induced arrhythmias and preserves connexin43 protein. Am J Physiol Heart Circ Physiol 2015; 308: H101-107.
[17] Dhein S, Seidel T, Salameh A, Jozwiak J, Hagen A, Kostelka M, Hindricks G, Mohr FW. Remodeling of cardiac passive electrical properties and susceptibility to ventricular and atrial arrhythmias. Front Physiol 2014; 5: 424.
[18] Shin SY, Jo WM, Min TJ, Kim BK, Song DH, Hyeon SH, Kwon JE, Lee WS, Lee KJ, Kim SW, Kim TH, Kim CJ, Im SI, Lim HE. Gap junction remodelling by chronic pressure overload is related to the increased susceptibility to atrial fibrillation in rat heart. Europace 2015; 17: 655-663.
[19] Siragam V, Cui X, Masse S, Ackerley C, Aafaqi S, Strandberg L, Tropak M, Fridman MD, Nanthakumar K, Liu J, Sun Y, Su B, Wang C, Liu X, Yan Y, Mendlowitz A, Hamilton RM. TMEM43 mutation p.S358L alters intercalated disc protein expression and reduces conduction velocity in arrhythmogenic right ventricular cardiomyopathy. PLoS One 2014; 9: e109128.
[20] Grippo AJ, Moffitt JA, Henry MK, Firkins R, Senkler J, McNeal N, Wardwell J, Scotti MA, Dotson A, Schultz R. Altered Connexin 43 and Connexin 45 protein expression in the heart as a function of social and environmental stress in the prairie vole. Stress 2015; 18: 107-114.
[21] Chang Y, Yu T, Yang H, Peng Z. Exhaustive exercise-induced cardiac conduction system injury and changes of cTnT and Cx43. Int J Sports Med 2015; 36: 1-8.
[22] Prudat Y, Kucera JP. Nonlinear behaviour of conduction and block in cardiac tissue with heterogeneous expression of connexin 43. J Mol Cell Cardiol 2014; 76: 46-54.
[23] Lubkemeier I, Bosen F, Kim JS, Sasse P, Malan D, Fleischmann BK, Willecke K. Human Connexin43E42K mutation from a sudden infant death victim leads to impaired ventricular activation and neonatal death in mice. Circ Cardiovasc Genet 2015; 8: 21-29.