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

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Abstract: Chronic arrhythmia is common in clinic. Shenxian shengmai 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\(^{+}\)-Mg\(^{2+}\)-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 protein expression 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 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-
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, Ca²⁺-Mg²⁺-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: SYXX-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 Ca²⁺-Mg²⁺-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 adenylyl 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 Ca²⁺-Mg²⁺-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 Ca²⁺-Mg²⁺-ATPase activities were detected according to the manual.
Shenxian shengmai oral liquid and chronic arrhythmia

Western blot

Total protein was separated by SDS-PAGE and transferred to PVDF membrane. After block-
ed 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 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. *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. *P < 0.05, compared with group C; †P < 0.05, compared with group A.
Shenxian shengmai oral liquid and chronic arrhythmia

Impact of shenxian shengmai oral liquid on Na⁺-K⁺-ATPase and Ca²⁺-Mg²⁺-ATPase levels in myocardium

Na⁺-K⁺-ATPase and Ca²⁺-Mg²⁺-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 autonomic 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 Ca²⁺-Mg²⁺-ATP is to active transport Ca²⁺ across the cell membrane to extracellular and absorb Ca²⁺ from cytoplasm to sarcoplasmic reticulum. Ca²⁺ release disorder in the calcium pool may cause sarcoplasmic reticulum permeability decline for Ca²⁺, leading to decreased calcium influx, slow down the pacemaker cell automatic depolarization in sinoatrial node, and induce chronic arrhythmia. Na⁺-K⁺-ATPase and Ca²⁺-Mg²⁺-ATPase both involved in myocardial cell membrane ion channel and ion trans-

Figure 4. The impact of shenxian shengmai oral liquid on Na⁺-Mg²⁺-ATPase and Ca²⁺-Mg²⁺-ATPase levels in myocardium. Group A. Model group; B. Shenxian shengmai oral liquid group; C. Control. △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).
Shenxian shengmai oral liquid and chronic arrhythmia

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 autorhythmcity, 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 KCNJ2 gene on chromosome 17. Kir2.1 protein mediated IK1 current 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 KCNJ2 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. Shenxian 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 KCNJ2 gene, and this needs further investigation.

Disclosure of conflict of interest

None.

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References


Shenxian shengmai oral liquid and chronic arrhythmia


