

Research Article

The Electrophysiological Effects of Qiliqiangxin on Cardiac Ventricular Myocytes of Rats

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Qiliqiangxin, a Chinese herb, represents the affection in Ca channel function of cardiac myocytes. It is unknown whether Qiliqiangxin has an effect on Na current and K current because the pharmacological actions of this herb's compound are very complex. We investigated the rational usage of Qiliqiangxin on cardiac ventricular myocytes of rats. Ventricular myocytes were exposed acutely to 1, 10, and 50 mg/L Qiliqiangxin, and whole cell patch-clamp technique was used to study the acute effects of Qiliqiangxin on Sodium current (I_{Na}), outward currents delayed rectifier outward K^+ current (I_K), slowly activating delayed rectifier outward K^+ current (I_{Ks}), transient outward K^+ current (I_{to}), and inward rectifier K^+ current (I_{K1}). Qiliqiangxin can decrease I_{Na} by $28.53\% \pm 5.98\%$, and its IC_{50} was 9.2 mg/L. 10 and 50 mg/L Qiliqiangxin decreased by $37.2\% \pm 6.4\%$ and $55.9\% \pm 5.5\%$ summit current density of I_{to} . 10 and 50 mg/L Qiliqiangxin decreased I_{Ks} by $15.51\% \pm 4.03\%$ and $21.6\% \pm 5.6\%$. Qiliqiangxin represented a multifaceted pharmacological profile. The effects of Qiliqiangxin on Na and K currents of ventricular myocytes were more profitable in antiarrhythmic therapy in the clinic. We concluded that the relative efficacy of Qiliqiangxin was another choice for the existing antiarrhythmic therapy.

1. Introduction

The traditional Chinese herbs have been proven safe and efficient in the management of some diseases including arrhythmia since ancient times. Presently, there are four main classes of antiarrhythmic agents in clinical therapy. Three classes of them act on ion channels. As for the Chinese herb—Qiliqiangxin—few know the mechanism of antiarrhythmia. How does it impact on ion channels? Qiliqiangxin capsule is the developed Chinese herbs, which includes over 11 ingredients such as Ginseng, Radix Astragali, Aconite Root, *Salvia miltiorrhiza*, and Semen Lepidii Apetali. It was proved to be effective and safe by phase 3 clinic trial for the treatment of patients with chronic heart failure [1, 2]. Qiliqiangxin capsule

also can improve heart function and decrease serum level of TNF- α and relieve inflammatory cell infiltration of myocardium in rats with adriamycin induced cardiomyopathy [3]. In our previous study, Qiliqiangxin affected L-type Ca^{2+} channel and blocked I_{Ca-L} , as well as affected cardiac function finally. Qiliqiangxin has biphasic action that is either class IV antiarrhythmic agent or the agent for treating chronic heart failure [4, 5]. It is unknown whether Qiliqiangxin has an effect on Na current and K current because the pharmacological actions of this herb's compound are very complex. In this research, we compared the acute effects of Qiliqiangxin on the inward currents I_{Na} and outward currents I_K , I_{Ks} , I_{to} , and I_{K1} of ventricular myocytes and hoped to demonstrate the rational usage of Qiliqiangxin.

2. Material and Methods

2.1. Vegetal Material. Qiliqiangxin consists of Ginseng, Radix Astragali, Aconite Root, *Salvia miltiorrhiza*, Semen Lepidii Apetali, Cortex Periplocae Sepii Radicis, Rhizoma Alismatis, *Carthamus tinctorius*, Polygonatum Odorati, Seasoned Orange Peel, and Ramulus Cinnamomi [3] (Yiling Pharmaceutical Corporation, Shijiazhuang, China). The drug powder dissolved with sterile water at the concentration of 2.67 g/mL. 1 mg/L, 10 mg/L, and 50 mg/L Qiliqiangxin were prepared for the study.

2.2. Study Models. A total of 26 healthy Sprague-Dawley rats (9–11 weeks old, either sex, weight 210 to 300 g) were used in the study. All the rats used in the following experiments were subject to the Guiding Principles for the Care and Use of Laboratory Animals and the Recommendations from the Declaration of Tongji University. Cardiac ventricular myocytes were isolated from the hearts of rats using previous protocols [6]. Briefly, hearts were rapidly excised and cycloperfused with low calcium Tyrode's solution containing 0.08% collagenase, 0.006% protease, and then we get a single ventricular myocyte. The single ventricular myocyte selected for study is rod shaped and had clear striations and smooth and glossy surface.

2.3. Whole Cell Patch Clamp. We recorded that Na current in the external solution contained (mmol/L) Choline-Cl 120, NaCl 25, CsCl 4, CaCl₂ 1.8, CoCl₂ 2, MgCl₂ 1, HEPES 10, and Glucose 10; pH was adjusted to 7.4 with CsOH. The pipette solution contained (mmol/L) CsCl 140, NaCl 10, HEPES 5, EGTA 5, and Na₂ATP 5; pH was adjusted to 7.3 with CsOH. I_{Na} was elicited from a potential -90 mV to +40 mV with 10 mV increments and by 200 ms pulses. For I_K recording, the external solution contained (mmol/L) Choline-Cl 145, MgCl₂ 2, EGTA 1, HEPES 5, and Glucose 5.5; pH was adjusted to 7.4 with LiOH. The pipette solution contained (mmol/L) KCl 140, MgCl₂ 1, K₂ATP 5, HEPES 5, and EGTA 10; pH was adjusted to 7.3 with KOH. For I_{K1} recording, the external solution contained (mmol/L) Choline-Cl 145, KCl 5, MgCl₂ 1, EGTA 5, HEPES 10, and Glucose 10; pH was adjusted to 7.4 with KOH. The pipette solution contained (mmol/L) KCl 140, CaCl₂ 1, EGTA 10, HEPES 5, and K₂ATP 5; pH was adjusted to 7.3 with KOH. For I_{to} recording, the external solution contained (mmol/L) NaCl 140, KCl 4, CaCl₂ 1.5, MgCl₂ 1, CdCl₂ 0.5, and HEPES 5, Glucose 10; pH was adjusted to 7.4 with NaOH. The pipette solution contained (mmol/L) KCl 140, MgCl₂ 1, K₂ATP 5, EGTA 5, and HEPES 10; pH was adjusted to 7.3 with KOH. All recordings are at room temperature. The external solution was filled with 95% O₂ and 5% CO₂. I_K was elicited from the holding potential of -50 mV to +60 mV and by 4500 ms pulses with 10 mV increments. I_{K1} was elicited from the holding potential of -120 mV to +60 mV and by 200 ms pulses with 10 mV increments. I_{to} was elicited from the holding potential of -80 mV and by 300 ms pulses with 10 mV increments from a potential -40 mV to +70 mV. The effects of Qiliqiangxin: we perfuse cell with Qiliqiangxin of 1, 10, 50 mg/L, and every cell was perfused 2-3 concentration

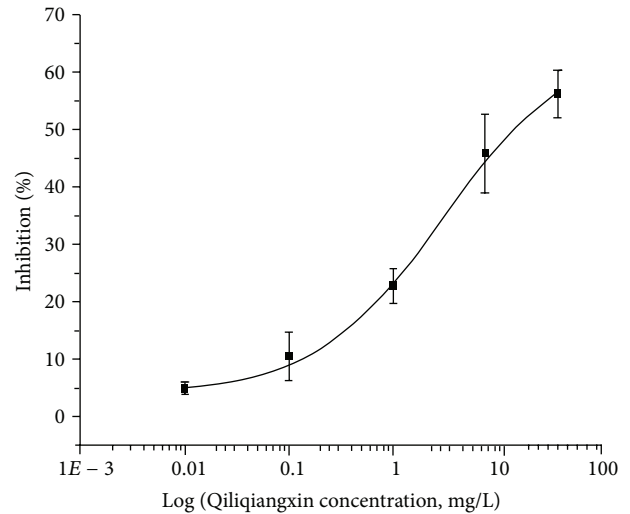


FIGURE 1: Qiliqiangxin's effect on I_{Na} of ventricular myocytes. Its IC_{50} was 9.2 mg/L.

steps. To normalize for differences in total membrane area, current densities (in pA/pF) were calculated by dividing the total current by the membrane capacitance of the cell. Data were sampled at 10 kHz and filtered at 2 kHz by using an Axopatch 200 A amplifier (Axon Instruments).

2.4. Statistical Analysis. pCLAMP 9.0 software was used for data acquisition, and analysis values are presented as means \pm S.D. Statistical comparisons between the different amiodarone concentrations groups were obtained by ANOVA. Comparisons between control and hypertrophied myocytes group means were performed with Student's *t*-test. Differences with $P < 0.05$ were considered significant, completed by SPSS 11.5 Statistically package. Concentration response relationships were fit to the Hill equation to determine the concentration of drug required for 50% inhibition (IC_{50}).

3. Result

3.1. Current Density. The inward currents density of I_{Na} in cardiac ventricular myocytes (pA/pF, $n = 10$) was -56.46 ± 4.88 . The outward current densities of I_K , I_{Ks} , I_{to} , and I_{K1} (pA/pF, $n = 8$) were 7.27 ± 0.95 , 5.68 ± 0.56 , 34.71 ± 2.83 , -22.82 ± 5.34 (I_{K1} inward), and 10.16 ± 0.20 (I_{K1} outward).

3.2. Effects of Qiliqiangxin on I_{Na} , I_{to} , I_{K1} , and I_K . 1 Mg/L Qiliqiangxin can decrease I_{Na} of ventricular myocytes by $28.53\% \pm 5.98\%$, and its IC_{50} was 9.2 mg/L (Figure 1). Hypoconcentration (1 mg/L) Qiliqiangxin had no effects on I_{to} on ventricular myocytes, while hyperconcentration Qiliqiangxin had inhibition action. 10 and 50 mg/L Qiliqiangxin decreased by $37.2\% \pm 6.4\%$ and $55.9\% \pm 5.5\%$ summit current density of I_{to} (Figure 2). For I_{K1} , the difference concentration of Qiliqiangxin had no effects on I_{K1} .

Delayed rectifier K⁺ current (I_K) is the major outward current responsible for ventricular repolarization. 1, 10, and

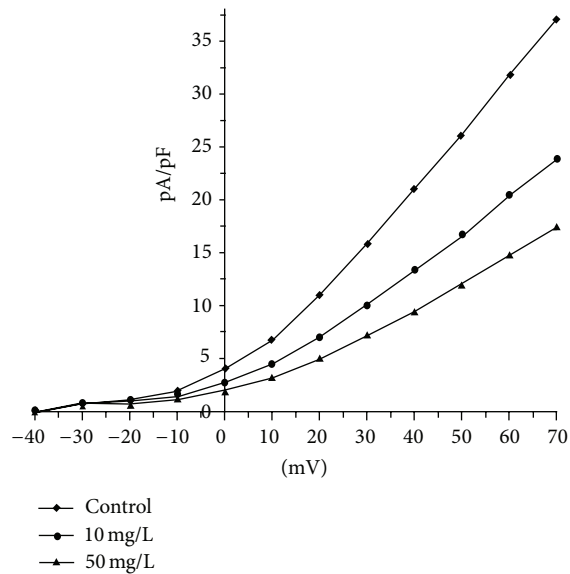


FIGURE 2: The effects of 10 and 50 mg/L Qiliqiangxin on current density-voltage curve of I_{to} in ventricular myocytes.

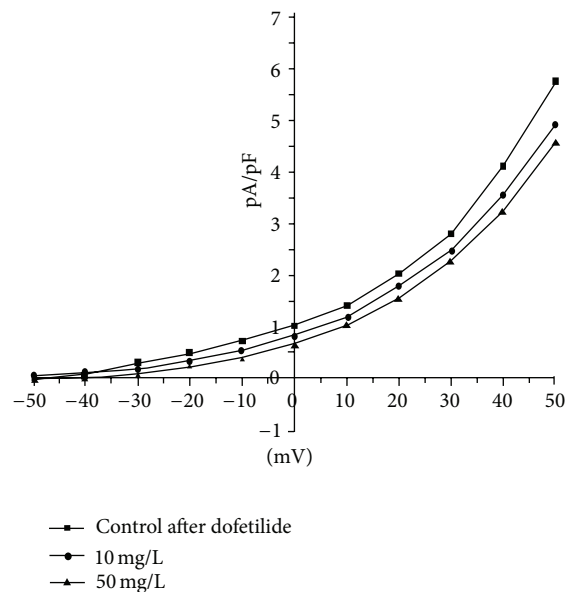


FIGURE 3: The effect of 10 and 50 mg/L Qiliqiangxin on current density-voltage curve of I_{Ks} in ventricular myocytes.

50 mg/L Qiliqiangxin decreased the current density of I_K by $20.98\% \pm 3.97\%$, $31.18\% \pm 5.1\%$, and $61.52\% \pm 5.97\%$. I_K comprises two distinct current components slowly activating delayed rectifier outward K^+ current (I_{Ks}) and rapidly activating delayed rectifier outward K^+ current (I_{Kr}). We used dofetilide, a class III antiarrhythmic agent and a selective blocker of I_{Kr} , to inhibit I_{Kr} and then I_{Ks} was recorded alone. 10 and 50 mg/L Qiliqiangxin decreased by $15.51\% \pm 4.03\%$ and $21.6\% \pm 5.6\%$ current density of I_{Ks} on ventricular myocytes (Figure 3).

4. Discussion

From our previous study, Qiliqiangxin has diphasic action that is either class IV antiarrhythmic agent or the agent for treating chronic heart failure [5]. Qiliqiangxin includes over 11 ingredients. The mechanism of the antiarrhythmic action is complex and not completely understood. It should be contacted with Radix Astragal, Aconite Root, and Shensongyangxin. Radix Astragal effectively protected against cardiac dysfunction [7]. Aconite Root was proved to have positive inotropic and chronotropic action [8]. Shensongyangxin capsule was reported to effectively block I_{Ca-L} [9]. There were four main classes of antiarrhythmic agents in Vaughan Williams' classification. Qiliqiangxin affected L-type Ca^{2+} channel and blocked I_{Ca-L} . It should be a class IV agent. Does Qiliqiangxin have an effect on other currents, such as Na current and K currents, because of its antiarrhythmic action? We had introduced Qiliqiangxin to test Na current and K currents. Interestingly, 1 mg/L Qiliqiangxin can decrease I_{Na} of ventricular myocytes by $28.53\% \pm 5.98\%$, and its IC_{50} was 9.2 mg/L. K currents are outward currents that repolarize the ventricular myocyte are numerous and complex and there is substantial interspecies variation in the profile of repolarizing currents. Prominent I_{to} currents have been recorded in ventricular myocytes isolated from the hearts of many species, including mice, rats, rabbits, cows, cats, dogs, ferrets and humans. In ventricular myocytes of rat, 10 and 50 mg/L Qiliqiangxin decreased by $37.2\% \pm 6.4\%$ and $55.9\% \pm 5.5\%$ summit current density of I_{to} . 1, 10, and 50 mg/L Qiliqiangxin decreased current density of I_K by $20.98\% \pm 3.97\%$, $31.18\% \pm 5.1\%$ and $61.52\% \pm 5.97\%$. 10 and 50 mg/L Qiliqiangxin decreased by $15.51\% \pm 4.03\%$ and $21.6\% \pm 5.6\%$ current density of I_{Ks} . Acute Qiliqiangxin application can inhibit I_{to} and I_K . 10 and 50 mg/L Qiliqiangxin had little effects on I_{Kr} , and the resting membrane potential was not affected by it. Qiliqiangxin represented class I and IV antiarrhythmic agents. It can prolong QT interval, but it does not have a similar deleterious effect as pure class III compounds. Therefore, Qiliqiangxin represented a multifaceted pharmacological profile.

During the last decade, antiarrhythmic strategies have changed dramatically. In general, class I agents have the potential to increase mortality in patients with significant structural heart disease even though they may suppress cardiac arrhythmias. Class III and IV agents did not decrease the outcomes in clinic obviously. Qiliqiangxin is as one developed Chinese herbs, which expressed the inhibition of I_{Na} , I_{Ca-L} , and multi K^+ channels. The effects of Qiliqiangxin on ventricular myocytes were more profit in clinic efficacy. It is the most promising drug because of the relative efficacy and safety. Even though the mechanism of the antiarrhythmic action of Qiliqiangxin is complex and not completely understood, Qiliqiangxin is another choice for antiarrhythmic therapy at least. As for the further action in clinic of this antiarrhythmic agent, we need more research in the future.

Authors' Contribution

Yidong Wei and Xiaoyu liu are co-first authors.

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References

- [1] Y. L. Wu, C. H. Gu, G. C. Xu, C. Wei, and X. D. Gao, "Clinical observation of randomized double-blind and multicenter trial on Qiliqiangxin capsule in the treatment of chronic heart failure," *Chinese Journal of Difficult and Complicated Cases*, vol. 6, pp. 55–58, 2007.
- [2] J. H. Ling, P. B. Wu, W. Y. Cai, X. B. Xu, M. R. Zhuang, and C. L. Li, "Therapeutic effect of Qiliqiangxin capsule on the patients with chronic congestive heart failure," *Chinese Journal of Difficult and Complicated Cases*, vol. 5, pp. 49–55, 2008.
- [3] H. Xiao, Y. Song, Y. Li, Y. Liao, and J. Chen, "Qiliqiangxin regulates the balance between tumor necrosis factor- α and interleukin-10 and improves cardiac function in rats with myocardial infarction," *Cellular Immunology*, vol. 260, no. 1, pp. 51–55, 2009.
- [4] D. M. Bers, "Calcium cycling and signaling in cardiac myocytes," *Annual Review of Physiology*, vol. 70, pp. 23–49, 2008.
- [5] Y. Wei, X. Liu, L. Hou, W. Che, E. The, and M. V. Jhummon, "Qiliqiangxin affects L type Ca^{2+} current in the normal and hypertrophied rat heart," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 131830, 4 pages, 2012.
- [6] J.-P. Benitah, A. M. Gomez, and P. Bailly, "Heterogeneity of the early outward current in ventricular cells isolated from normal and hypertrophied rat hearts," *The Journal of Physiology*, vol. 469, pp. 111–138, 1993.
- [7] Z. Zhao, W. Wang, and F. Wang, "Effects of astragaloside IV on heart failure in rats," *Chinese Medicine*, vol. 4, article 6, 2009.
- [8] J. Lin, C. Chan, C. Yang, Y. Wang, H. Chiou, and Y. Su, "A cardiotoxic Chinese herb, a new medical treatment choice for portal hypertension?" *Experimental Biology and Medicine*, vol. 232, no. 4, pp. 557–564, 2007.
- [9] N. Li, Y. Huo, K. Ma, Q. Sun, and J. Pu, "Effects of solution of dry powder of ShenSongYangXin capsule on sodium current and L-type calcium current in ventricular myocytes: experiment with guinea pig," *Zhonghua Yi Xue Za Zhi*, vol. 87, no. 14, pp. 995–998, 2007.