

Research Article

Qiliqiangxin Affects L Type Ca^{2+} Current in the Normal and Hypertrophied Rat Heart

Yidong Wei,¹ Xiaoyu Liu,² Lei Hou,¹ Wenliang Che,¹ Erlinda The,¹
and Muktanand Vikash Jhummon¹

¹ Department of Cardiology, Shanghai Tenth People's Hospital of Tongji University, 301 Yanchang Road, Shanghai 200072, China

² Department of Traditional Chinese Medicine, Shanghai Tenth People's Hospital of Tongji University, 301 Yanchang Road, Shanghai 200072, China

Correspondence should be addressed to Yidong Wei, camelheart@163.com

Received 24 November 2011; Accepted 19 January 2012

Academic Editor: Hao Xu

Copyright © 2012 Yidong Wei et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Qiliqiangxin capsule is newly developed Chinese patent drug and proved to be effective and safe for the treatment of patients with chronic heart failure. We compared the effects of different dose Qiliqiangxin on L type Ca^{2+} current ($I_{\text{Ca-L}}$) between normal and hypertrophied myocytes. A total of 40 healthy Sprague—Dawley rats were used in the study. The rats were randomly divided into two groups (control group and hypertrophy group). Cardiac hypertrophy was induced by pressure overload produced by partial ligation of the abdominal aorta. The control group was the sham-operated group. After 1 month, cardiac ventricular myocytes were isolated from the hearts of rats. Ventricular myocytes were exposed to 10 and 50 $\mu\text{mol/L}$ Qiliqiangxin, and whole cell patch-clamp technique was used to study the effects of Qiliqiangxin on $I_{\text{Ca-L}}$. The current densities of $I_{\text{Ca-L}}$ were similar in control group ($-12.70 \pm 0.53 \text{ pA/pF}$, $n = 12$) and in hypertrophy group ($-12.39 \pm 0.62 \text{ pA/pF}$, $n = 10$). They were not statistically significant. 10 and 50 $\mu\text{mol/L}$ Qiliqiangxin can decrease $I_{\text{Ca-L}}$ peak current $48.6\% \pm 16.8\%$ and $59.0\% \pm 4.4\%$ in control group. However, the peak current was only reduced $16.73\% \pm 8.03\%$ by 50 $\mu\text{mol/L}$ Qiliqiangxin in hypertrophied myocytes. The inhibited action of Qiliqiangxin on $I_{\text{Ca-L}}$ of hypertrophy group was lower than in control group. Qiliqiangxin affected L-type Ca^{2+} channel and blocked $I_{\text{Ca-L}}$, as well as affected cardiac function finally. Qiliqiangxin has diphasic action that is either class IV antiarrhythmic agent or the agent of effect cardiac function.

1. Introduction

The traditional Chinese medicines have proven the safety and efficiency of herbs in the management of some diseases since ancient times. Qiliqiangxin capsule is newly developed Chinese patent drug and proved to be effective and safe by phase 3 clinic trial for the treatment of patients with chronic heart failure. It includes over 11 ingredients such as Ginseng, Radix Astragali, Aconite Root, Salvia Miltiorrhiza, and Semen Lepidii Apetali [1, 2]. Previous studies showed Qiliqiangxin capsule can improve heart function and decrease serum level of $\text{TNF-}\alpha$ and relieve inflammatory cell infiltration of myocardium in rats with adriamycin induced cardiomyopathy [3]. Nevertheless, the excitation-contraction coupling in the cardiac myocyte is triggered by

the influx of Ca^{2+} through L type Ca^{2+} channel and inducing Ca^{2+} release from the sarcoplasmic reticulum [4, 5]. Blocking Ca^{2+} channel and reducing Ca^{2+} overload will be of benefit in the progress of heart failure. Qiliqiangxin should affect L type Ca^{2+} channel and further alter cellular Ca^{2+} regulation as well as effect the heart function finally. We compared the effects of different dose Qiliqiangxin on L type Ca^{2+} current ($I_{\text{Ca-L}}$) between normal and hypertrophied myocytes and to comprehend the rational usage of Qiliqiangxin on hypertrophied myocytes in this study.

2. Material and Methods

2.1. Vegetal Material. Qiliqiangxin consists of Ginseng, Radix Astragali, Aconite Root, Salvia Miltiorrhiza, Semen

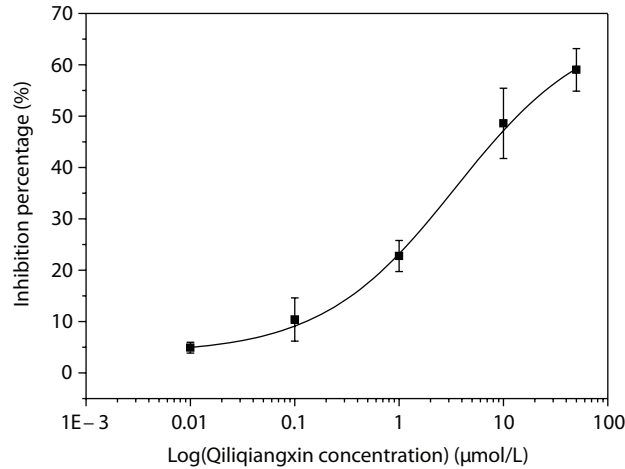


FIGURE 1: The concentration-dependent manner of Qiliqiangxin on I_{Ca-L} in cardiac myocytes of rat ($IC_{50} = 10.38 \mu\text{mol/L}$).

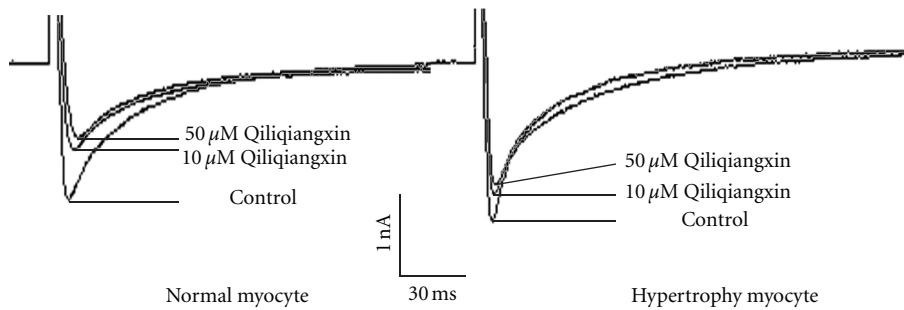


FIGURE 2: The effects of 10 and 50 $\mu\text{mol/L}$ Qiliqiangxin on I_{Ca-L} in normal and hypertrophied myocytes.

Lepidii Apetali, Cortex Periplocae Sepii Radicis, Rhizoma Alismatis, Carthamus Tinctorius, Polygonatum Odorati, Seasoned Orange Peel, and Rumulus Ginnamomi [3] (Yiling Pharmaceutical Corporation, Shijiazhuang, China). The drug powder was dissolved with sterile water at the concentration of 2.67 g/mL. 10 $\mu\text{mol/L}$ and 50 $\mu\text{mol/L}$ Qiliqiangxin were prepared for the study.

2.2. Study Models. A total of 40 healthy Sprague-Dawley rats (9–11-week 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. The rats were randomly divided into two groups (control group and hypertrophy group). Cardiac hypertrophy was induced by pressure overload produced by partial ligation of the abdominal aorta by using the method described by Anderson [6–8]. The control group was the sham-operated group; the aorta was dissected without application of the ligation. After operation, both groups were fed up with normal fodder and tap water in different cages for one month.

2.3. Cardiac Ventricular Myocytes Isolation. Cardiac ventricular myocytes were isolated from the hearts of rats using previous protocols [9]. Briefly, hearts were rapidly

excised and cycloperfused with low calcium Tyrode's solution containing 0.08% Collagenase, 0.006% Protease, and then get single ventricular myocyte. The single ventricular myocyte selected for study is rod shaped, had clear striations and smooth and glossy surface.

2.4. Whole Cell Patch Clamp. We recorded Ca^{2+} current in a Na^{+} -free bath solution. To block outward K^{+} currents the bath contained (mM): 120 CsCl, 2 CaCl_2 , 10 TEA, 5 4-AP, 1 MgCl_2 , 5 HEPES, 5 Glucose. PH = 7.4 (CsOH). The patch pipettes (borosilicate glass, 1.5–3 M Ω) were filled with the pipette solution (mM): 120 CsCl, 1 CaCl_2 , 10 HEPES, 5 Mg-ATP, 10 EGTA. PH = 7.2 (CsOH). All recordings are at room temperature. The external solution was filled with 95% O_2 and 5% CO_2 . Ca^{2+} currents were elicited by voltage steps from -90 to $+55$ mV. Compensated series resistance was 1.59 ± 0.20 M Ω . Cell capacitance averaged 26.9 ± 4.1 pF ($n = 10$ per group). 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 200A amplifier (Axon Instruments).

2.5. Statistical Analysis. pCLAMP 9.0 software was used for data acquisition and analysis values are presented as

TABLE 1: The measurement of rats basic characteristics.

	BW (g)	HW (mg)	LVW (mg)	HW/BW (mg/g)	LVW/BW (mg/g)
Control	237 ± 23	730 ± 26	507 ± 48	2.67 ± 0.10	2.01 ± 0.15
Hypertrophy	229 ± 18	810 ± 15*	672 ± 50*	3.43 ± 0.15*	2.63 ± 0.19*

Notation. * $P < 0.05$, compared to control group. BW: body weight, HW: heart weight, left ventricular weight: LVW.

means ± 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. Results

3.1. Rats Characteristics. The rat hearts were significantly larger in hypertrophy group (810 ± 15 mg, $n = 22$) than in control group (730 ± 26 mg, $n = 18$). However, there was no difference in body weight between the two groups. Heart weight index (heart weight/body weight, HW/BW) and left ventricular weight index (left ventricular weight/body weight, LVW/BW) in hypertrophy group were greater than those in control group. They were statistically significant (Table 1).

3.2. Effects of Qiliqiangxin on I_{Ca-L} . The current densities of I_{Ca-L} were similar in control group (-12.70 ± 0.53 pA/pF, $n = 12$) and in hypertrophy group (-12.39 ± 0.62 pA/pF, $n = 10$). They were not statistical significant. Qiliqiangxin obviously decrease I_{Ca-L} of normal myocytes and represented a concentration-dependent manner. Its IC_{50} was $10.38 \mu\text{mol/L}$ (Figure 1). 10 and $50 \mu\text{mol/L}$ Qiliqiangxin can decreased I_{Ca-L} peak current $48.6\% \pm 16.8\%$ and $59.0\% \pm 4.4\%$ in control group. Interestingly, I_{Ca-L} represented insensitivity for Qiliqiangxin in hypertrophied myocytes. The peak current was only reduced $16.73\% \pm 8.03\%$ by $50 \mu\text{mol/L}$ Qiliqiangxin. Therefore, the inhibited action of Qiliqiangxin on I_{Ca-L} of hypertrophy group was lower than in control group (Figure 2).

4. Discussion

Cardiac hypertrophy is associated with a significantly increased risk of cardiovascular morbidity and mortality that were frequently induced by electrical remodeling and arrhythmogenesis. The antiarrhythmic research was most based on normal myocytes, and whether they have same action on pathosis myocytes was unknown. As a result, means of treating hypertrophy-associated arrhythmias remain disappointingly ineffective. So far, there were four main classes of antiarrhythmic agents. Class IV agents are slow calcium channel blockers and decrease conduction through the AV node. They shorten the plateau of the action potential and reduce the contractility of the heart. Class IV

agents may be inappropriate in cardiac hypertrophy treatment. Nevertheless, blocking Ca^{2+} channels and reducing Ca^{2+} overload will be of benefit in the progress of cardiac hypertrophy.

In pressure overload hypertrophy models, we found that the currents amplitude of I_{Ca-L} on hypertrophied myocytes were higher than those in control. But the current densities were similar because of the swelling volume of hypertrophied myocytes. Acute application of Qiliqiangxin does inhibit I_{Ca-L} in normal cardiac myocytes. IC_{50} was $10.38 \mu\text{mol/L}$. 10 and $50 \mu\text{mol/L}$ Qiliqiangxin can, respectively, decreased $48.6\% \pm 16.8\%$ and $59.0\% \pm 4.4\%$ of the peak current of I_{Ca-L} in control group. To compare with the hypertrophy group, I_{Ca-L} showed different effects of Qiliqiangxin. Interestingly, the peak current was only reduced $16.73\% \pm 8.03\%$ by $50 \mu\text{mol/L}$ Qiliqiangxin. The inhibited action of Qiliqiangxin on I_{Ca-L} of hypertrophy group was lower than in control group. In other words, I_{Ca-L} represented more insensitivity for Qiliqiangxin in hypertrophied cardiac myocytes. Qiliqiangxin displayed the insensitiveness that may be facilitated for its utilization in cardiac hypertrophy and heart failure. Because it partly blocked I_{Ca-L} and did not weaken myocardial contractility basically. $10 \mu\text{mol/L}$ Qiliqiangxin obviously decreased $48.6\% \pm 16.8\%$ of the peak current of I_{Ca-L} in normal cardiac myocytes, which made it reserve antiarrhythmic activity as class IV agents. That also signifies we should deal with difference between hypertrophied heart and normal heart when we use Qiliqiangxin in the clinic.

Qiliqiangxin includes over 11 ingredients. The mechanism of the antiarrhythmic action is complex and not completely understood. It is hard to prove which herb has mainly contributed to the effect on L type Ca^{2+} channel. Recently, ShenSongYangXin capsule, a traditional Chinese herb, has been reported to effectively block I_{Ca-L} [10]. Zhao et al. had reported that Radix Astragali effectively protected against cardiac dysfunctional and morphological aberrations in experimental myocardial infarction [11]. Aconite Root was proved to have positive inotropic, positive chronotropic, vasodilation, and diuretic effects in the management of congestive heart failure [12]. Qiliqiangxin is composed of Radix Astragal, Aconite Root and parts of Shensong Yangxin which are the main active constituents of Qiliqiangxin. In the cardiac function, the excitation-contraction coupling of cardiac myocyte is triggered by Ca^{2+} influx through L-type Ca channels [4, 5]. Ca^{2+} influx activates calmodulin kinase that may activate transcription factors and cAMP response element binding protein (CREB). CREB promoted several cytokine secretions such as interleukin-10 (IL-10) [13, 14]. Inflammatory cytokines mainly derived from cardiac myocytes were involved in the progression of heart failure [15]. A proinflammatory cytokine (TNF- α) has been

linked to accelerate myocardial necrosis and deteriorated cardiac function. Serum level of TNF- α in patients with chronic heart failure increased and correlated with poor cardiac performance [16]. IL-10 and TNF- α induced left ventricular remodeling and dysfunction in the failing heart [17]. Qiliqiangxin may improve cardiac function of rats with MI through regulation the balance between TNF- α and IL-10 [3]. Blocking Ca²⁺ channels and reducing Ca²⁺ influx as well as weakening myocardial contractility will be the key point in the progress of cardiac hypertrophy and heart failure. The mechanism underlying the beneficial effects of Qiliqiangxin may involve the regulation of Ca²⁺ channel and reduce Ca²⁺ influx. Meantime, it influences several cytokine secretions indirectly. We concluded Qiliqiangxin affected L-type Ca²⁺ channel and blocked I_{Ca-L} , as well as affected cardiac function finally. Qiliqiangxin has diphasic action that is either class IV antiarrhythmic agent or the agent of effect cardiac function.

4.1. Study Limitations. This study was focused on the effect of Qiliqiangxin in I_{Ca-L} . Further studies should be on the regulation of Na⁺ and K⁺ channels.

Authors' Contribution

Y. Wei and X. Liu are cofirst authors.

Acknowledgment

This work was supported by the National Natural Science Funds of China (no. 30800466) for Y. Wei.

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] K. M. Dibb, H. K. Graham, L. A. Venetucci, D. A. Eisner, and A. W. Trafford, "Analysis of cellular calcium fluxes in cardiac muscle to understand calcium homeostasis in the heart," *Cell Calcium*, vol. 42, no. 4-5, pp. 503–512, 2007.
- [6] P. G. Anderson, M. F. Allard, and G. D. Thomas, "Increased ischemic injury but decreased hypoxic injury in hypertrophied rat hearts," *Circulation Research*, vol. 67, no. 4, pp. 948–959, 1990.
- [7] P. G. Anderson, S. P. Bishop, and S. B. Digererness, "Transmural progression of morphologic changes during ischemic contracture and reperfusion in the normal and hypertrophied rat heart," *American Journal of Pathology*, vol. 129, no. 1, pp. 152–167, 1987.
- [8] Y. Wei and J. Wei, "The effect of Amiodarone on K channel current in the normal and hypertrophied rat heart," *Journal of Health Science*, vol. 54, no. 5, pp. 529–534, 2008.
- [9] J. P. Bénitah, 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.
- [10] N. Li, Y. P. Huo, K. J. Ma, Q. Sun, and J. L. 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.
- [11] Z. Zhao, W. Wang, and F. Wang, "Effects of Astragaloside IV on heart failure in rats," *Chinese Medicine*, vol. 4, article 6, 2009.
- [12] J. S. Lin, C. Y. Chan, C. Yang et al., "A cardiogenic Chinese herb, a new medical treatment choice for portal hypertension," *Experimental Biology and Medicine*, vol. 232, no. 4, pp. 557–564, 2007.
- [13] C. Platzer, E. Fritsch, T. Elsner, M. H. Lehmann, H. D. Volk, and S. Prösch, "Cyclic adenosine monophosphate-responsive elements are involved in the transcriptional activation of the human IL-10 gene in monocytic cells," *European Journal of Immunology*, vol. 29, no. 10, pp. 3098–3104, 1999.
- [14] B. Samten, S. T. Howard, S. E. Weis et al., "Cyclic AMP response element binding protein positively regulates production of IFN- γ by T cells in response to a microbial pathogen," *The Journal of Immunology*, vol. 174, no. 10, pp. 6357–6363, 2005.
- [15] L. Gullestad and P. Aukrust, "The cytokine network in heart failure: pathogenetic importance and potential therapeutic targets," *Heart Fail Monitor*, vol. 2, no. 1, pp. 8–13, 2001.
- [16] M. Satoh, M. Nakamura, and H. Saitoh, "Tumor necrosis factor- α -converting enzyme and tumor necrosis factor- α in human dilated cardiomyopathy," *Circulation*, vol. 99, no. 25, pp. 3260–3265, 1999.
- [17] G. Torre-Amione, S. Kapadia, J. Lee et al., "Tumor necrosis factor- α and tumor necrosis factor receptors in the failing human heart," *Circulation*, vol. 93, pp. 704–711, 1996.

