Research Article

Qiliqiangxin Capsule Modulates Calcium Transients and Calcium Sparks in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

Yuxin Li,[1] Zhang Zhang,[1] Xuezeng Hao,[2] Tingting Yu,[1] and Sen Li [1]

[1]*School of Life Sciences, Beijing University of Chinese Medicine, Beijing, China*
[2]*Dongzhimen Hospital of Beijing University of Chinese Medicine, Beijing, China*

Correspondence should be addressed to Sen Li; senli@bucm.edu.cn

Background. The therapeutic effects of Qiliqiangxin capsule (QLQX), a Chinese patent medicine, in patients with chronic heart failure are well established. However, whether QLQX modulates cardiac calcium (Ca^{2+}) signals, which are crucial for the heart function, remains unclear. *Aim of the Study*. This study aimed to evaluate the role of QLQX in modulating Ca^{2+} signals in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). *Materials and Methods*. Fluorescence imaging was used to monitor Ca^{2+} signals in the cytosol and nuclei of hiPSC-CMs. For Ca^{2+} spark measurements, the line-scan mode of a confocal microscope was used. *Results*. The QLQX treatment substantially decreased the frequency of spontaneous Ca^{2+} transients, whereas the amplitude of Ca^{2+} transients elicited by electrical stimulation did not change. QLQX increased the Ca^{2+} spark frequency in both the cytosol and nuclei without changing the sarcoplasmic reticulum Ca^{2+} content. Interestingly, QLQX ameliorated abnormal Ca^{2+} transients in CMs differentiated from hiPSCs derived from patients with long-QT syndrome. *Conclusions*. Our findings provide the first line of evidence that QLQX directly modulates cardiac Ca^{2+} signals in a human cardiomyocyte model.

1. Introduction

Calcium is an important second messenger that participates in several physiological processes [1, 2]. In cardiomyocytes, extracellular calcium ion influx during action potentials promotes calcium release from the sarcoplasmic reticulum (SR) to the cytosol. This phenomenon is called calcium-induced calcium release and is essential for cardiac contraction [3]. Calcium in the cytoplasm flows into the nucleus through the nuclear pore complex (NPC), which regulates gene transcription. Many drugs modulate myocardial function through calcium signaling. For example, stachydrine hydrochloride targets calcium-handling proteins such as ryanodine receptors (RyRs) and phospholamban (PLN) [4]. Shengmai powder, a traditional Chinese medicine, regulates calcium signals to treat heart failure and angina pectoris [5].

The Qiliqiangxin capsule (QLQX) is a Chinese patent medicine composed of 11 herbs, including *Astragalus membranaceus*, ginseng, and *Aconitum carmichaelii* [6, 7]. Several clinical trials have shown that QLQX improves cardiac function and reduces mortality in patients with chronic heart failure (CHF) [6, 8, 9]. In rats, QLQX ameliorates cardiac remodeling caused by myocardial infarction [7, 10]. However, the effect of QLQX on cardiac calcium signals, which is crucial for heart function, remains unclear. Moreover, the recent mechanistic studies of QLQX are mainly based on animal models or animal cells. Because of species differences, experimental results in animal models cannot fully reflect the action of drugs in humans [11]. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been used as a source of human cardiomyocytes for drug testing, and normal (without disease modeling) hiPSC-CMs can be used to investigate the mechanism of action of drugs [12]. Therefore,

we used hiPSC-CMs to study the effect of QLQX on cardiac cytosolic and nuclear calcium signals.

2. Methods

2.1. Cells and Reagents. Normal and long-QT (KCNQ1, exon4, c.656 *G* > *A*) hiPSC-CMs were obtained from HELP Therapeutics (Nanjing, China) and cultured as described previously [13]. QLQX was obtained from Shijiazhuang Yiling Pharmaceutical Co., Ltd. (Hebei, China). According to the "Chinese Pharmacopoeia" and the relevant standards of the National Medical Products Administration, the quality standard of the QLQX capsules is stated as follows: HPLC determination of *Astragalus* by astragaloside IV (C41H68O14) shall be no less than 0.12 mg. The prescription is Astragalus 450 g, ginseng 225 g, Heishun tablets 112.5 g, Salvia 225 g, Tinglizi 150 g, Alisma 225 g, Polygonatum odoratum 75 g, cinnamon 90 g, safflower 90 g, fragrant Jiapi 180 g, and tangerine peel 75 g. The Tyrode solution contained 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM D-glucose, buffered with 10 mM HEPES at pH 7.4.

2.2. Cell Counting Kit-8 Assay (CCK-8). The viability of hiPSC-CMs treated with various QLQX concentrations were examined using a CCK-8kit (Melone, China) by following the manufacturer's protocol. A plate reader (SpectraMax i3X, Molecular Devices, CA, USA) was used to measure absorbance at 450 nm.

2.3. Flow Cytometry. The hiPSC-CMs were fixed in 4% paraformaldehyde (PFA) at room temperature for 15 min, and the cell membranes were permeabilized with 90% precooled methanol for 30 min on ice. The permeabilized cells were incubated with the cTnT primary antibody (Abcam, UK) and an appropriate secondary antibody. A CytoFLEX flow cytometer was used to analyze the percentage of cTnT-positive cells.

2.4. Immunostaining. The hiPSC-CMs were fixed in 4% PFA for 20 min and then permeabilized with 0.2% Triton X-100 for 10 min. After blocking with 1% bovine serum albumin (BSA)-phosphate buffered saline (PBS) for 15 min, the samples were incubated with the cTnT antibody (Abcam) overnight at 4°C. The Alexa Fluor-555 anti-mouse IgG (Invitrogen, Carlsbad, CA, USA) was used as the secondary antibody, and the nuclei were labeled with DAPI (Solarbio, China). A confocal microscope (FV3000; Olympus, Japan) was used for imaging. We used an ER-tracker to examine the structure of the nucleoplasmic reticulum (NR) as previously described [14]. The nuclei of living cells were labeled with Hoechst 33342 and images were captured using a confocal microscope.

2.5. Calcium Imaging. Fluorescence imaging of Fluo-4 (Invitrogen) was used to monitor calcium signals in the cytoplasm and nucleus. The cells were incubated in a Tyrode solution containing 5 μM Fluo-4-AM at 37°C for 30 min. The samples were then transferred to an inverted microscope. For local calcium signals, the confocal line-scan mode was employed, and the scanning lines were placed crossing the long axis of the cell so that the nucleus was approximately in the middle of the scanning line. Sparkmaster [15] and Peakcaller [16] were used to analyze the calcium signals.

2.6. High-Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC-MS/MS). To identify the compounds in QLQX using HPLC-MS/MS, QLQX was centrifuged at 12000 rpm for 10 min at 4°C, and the supernatant was diluted 2–100 times. The internal standard was passed through a 0.22 μm PTPE filter, and metabolite quantitative analysis was performed. The analysis platform (Ultimate 3000LC, Q Exactive HF, Thermo Fisher Scientific, USA) was employed, and separation was achieved using a Zorbax Eclipse C18 column (1.8 μm, 2.1 × 100 mm). The chromatographic separation conditions were column temperature, 30°C; flow rate, 0.3 mL/min; injection volume, 2 μL; and autosampler temperature, 4°C. Gradient elution with mobile phase compositions A (water + 0.1% formic acid) and B (acetonitrile) was performed. Data analysis was performed using the Compound Discoverer 3.2 software.

2.7. Statistical Analysis. Statistical tests were performed using the SPSS software, and statistical significance was set at $P < 0.05$. All data are expressed as the mean ± standard error (SE).

3. Results

Cardiomyocytes derived from hiPSCs stained positive for cardiac troponin *T* (cTnT), a cardiac-specific protein (Figure 1(a)). The flow cytometry analysis also indicated that 94.29% of hiPSC-CMs expressed cTnT (Figure 1(b)). hiPSC-CM viability was not substantially altered by treatment with QLQX at a range of concentrations (9, 27, 83, and 250 μg/ml), indicating that QLQX was not toxic to cardiomyocytes at the examined concentrations (Figure 1(c)).

As shown in Figure 2(a), the frequency of spontaneous Ca^{2+} transients was remarkably decreased by the QLQX treatment in hiPSC-CMs, together with elevated calcium transient amplitude, rise time, and full duration at half maximum (Figure 2(b)). In CMs differentiated from hiPSCs derived from patients with long-QT syndrome, abnormal Ca^{2+} transients could be observed, which were presumably caused by long trains of early afterdepolarizations [17]. QLQX effectively attenuated Ca^{2+} signal abnormalities (Figure 2(c)). Next, we investigated the potential role of QLQX in modulating electrical stimulation-elicited Ca^{2+} transients in normal hiPSC-CMs. The results indicated that the amplitude of these Ca^{2+} transients was not significantly changed by the QLQX application (Figure 3). We then studied the effect of QLQX on SR Ca^{2+} content in hiPSC-CMs, and the results suggested a similar amplitude but substantially decreased rise time of caffeine-induced Ca^{2+} rise, indicating an unchanged SR Ca^{2+} content but increased
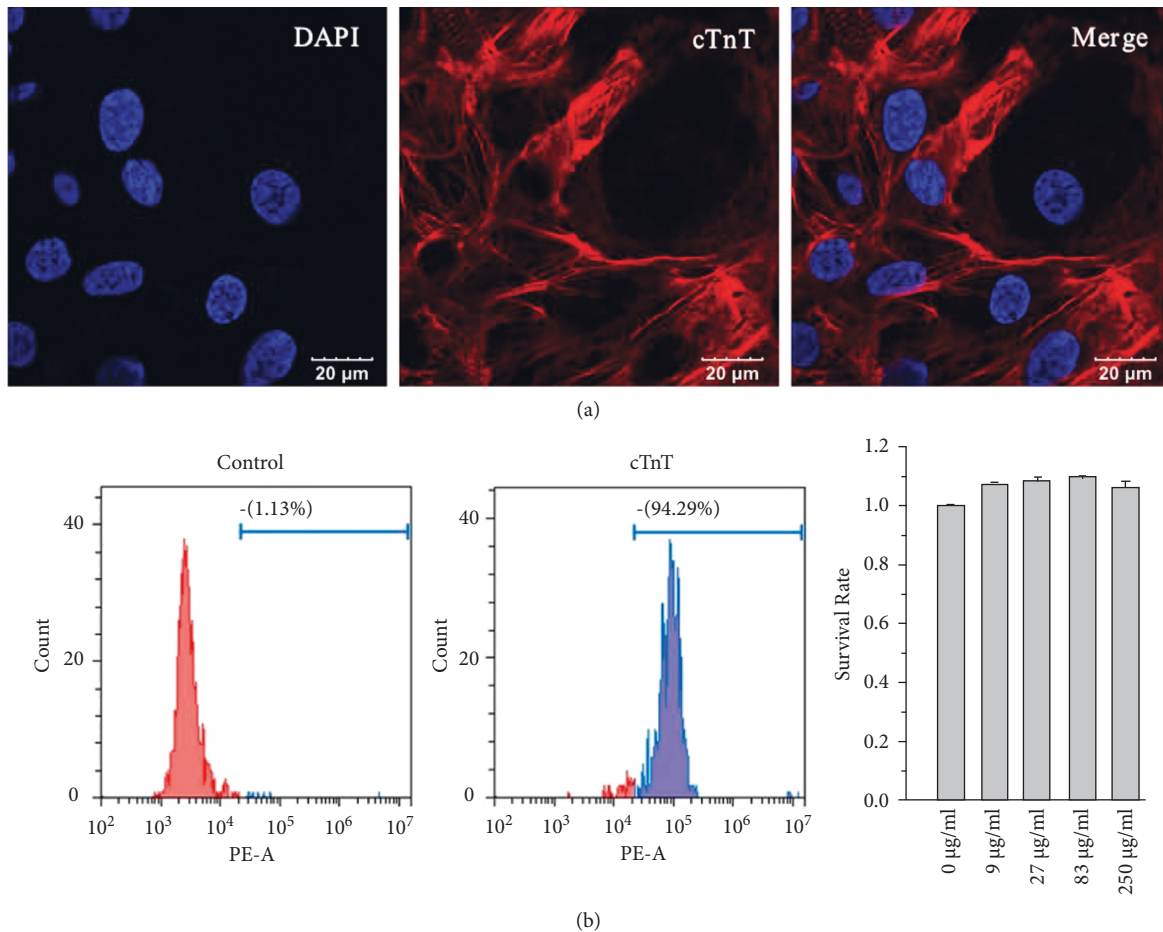
(a)

(b)

FIGURE 1: The expression of cTnT was examined by immunostaining (a) and flow cytometry analysis (b). Various concentrations of QLQX did not alter cell survival rate as examined by CCK-8 (c).

RyR activity (Figure 4). Consistently, Ca^{2+} spark measurements using confocal microscopy in line scanning mode indicated that QLQX increased the Ca^{2+} spark frequency in both the cytosol and nuclei (Figure 5). As the structural basis of nuclear Ca^{2+} sparks, the NR of hiPSC-CMs revealed by the ER-tracker loading, was not substantially changed by QLQX (Figure 6(a)). Moreover, nuclear Ca^{2+} waves in hiPSC-CMs displayed delayed kinetics compared to those in the cytosol, which were also not altered by the QLQX treatment (Figure 6(b)).

To better understand the mechanisms of action of QLQX, its chemical composition was analyzed using HPLC-MS/MS, and 62 components, such as cinnamic acid, flavonoids, astragalus, coumarin, and its derivatives were identified (Supplementary Table 1).

4. Discussion

The therapeutic effects of QLQX have been observed in several clinical studies [18–20]. In CHF patients, the combined use of the QLQX capsules with standard treatment further reduces the level of N-terminal pro-B-type natriuretic peptide (NT-proBNP) [9], a marker of heart failure status [21]. A meta-analysis of 129 clinical trials indicated

that QLQX combined with conventional treatment reduced the occurrence of major cardiovascular events and rehospitalization rates. Moreover, improvement in myocardial function without serious adverse events has been observed [20]. Mechanistic studies of QLQX have mainly been based on animal models. For example, QLQX ameliorates ventricular remodeling and improves cardiac function in a zebrafish heart failure model [22]. Furthermore, QLQX protected mice against damage caused by myocardial infarction induced by left coronary artery ligation. *In vitro* experiments have also shown that QLQX attenuates hypoxia-induced apoptosis in cardiomyocytes [23]. In normal and hypertrophic rat cardiomyocytes, QLQX inhibited $I_{Ca,L}$, which may serve as one of the underlying mechanisms of its therapeutic effects [24]. A recent study investigated the protective effect of QLQX on doxorubicin-induced cardiotoxicity in a rat model [25]. Left ventricular remodeling is also alleviated by QLQX in rats with pressure overload-induced heart failure [26].

hiPSC-CMs have been widely used as a source of human cardiomyocytes for evaluating the effects of drugs on cardiac function. For example, field stimulation-induced contraction and Ca^{2+} transients of hiPSC-CMs have been employed as key indicators of drug-induced changes in myocardial
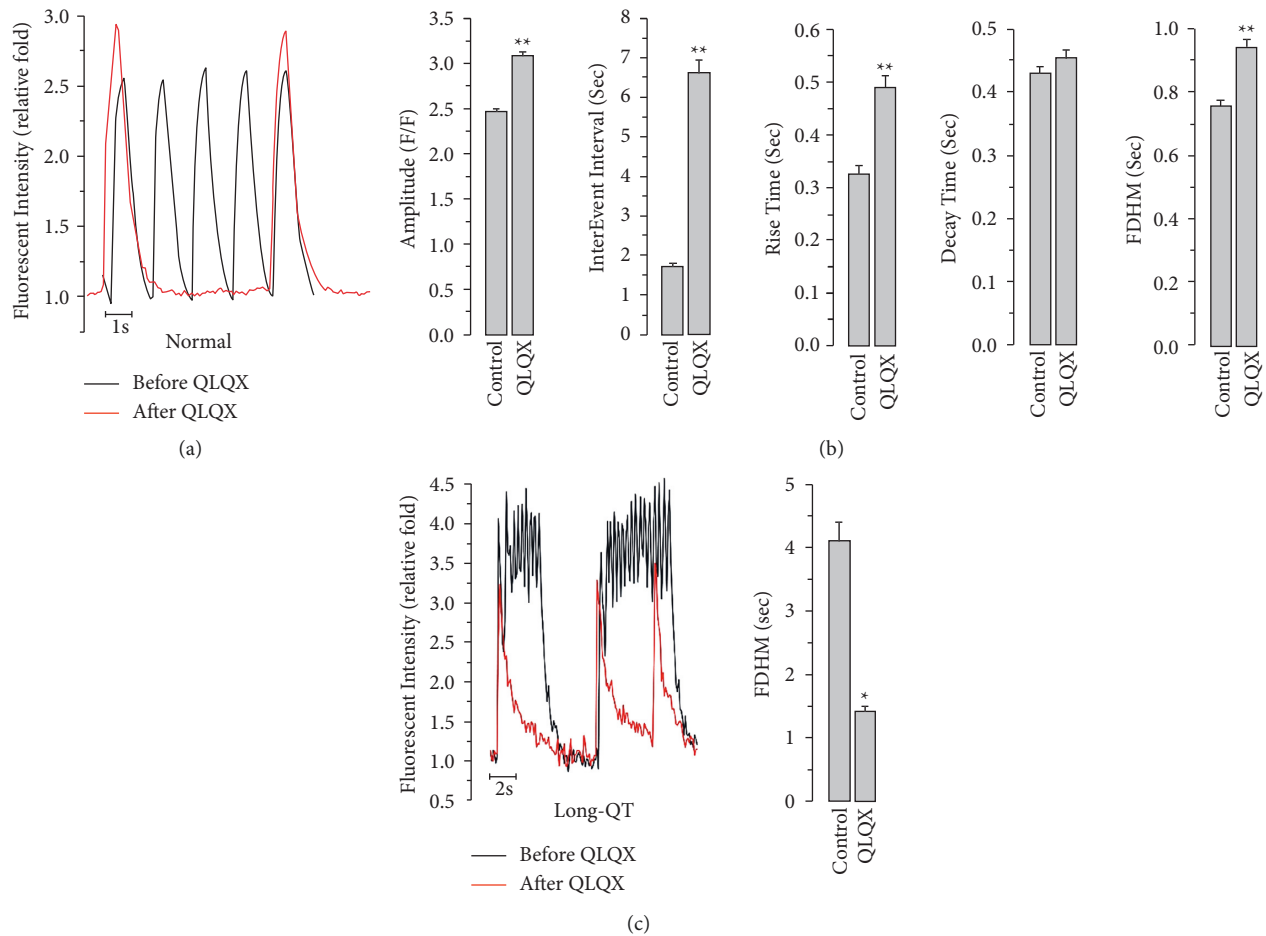
(a)

(b)

(c)

FIGURE 2: The effects of QLQX on spontaneous Ca^{2+} transients in hiPSC-CMs. (a) Representative Ca^{2+} transients before and after the QLQX application (83 μg/ml, 3 minutes). (b) The QLQX treatment significantly decreased the frequency but increased the amplitude, rise time, and full duration at half maximum (FDHM) of spontaneous Ca^{2+} transients. $n = 48$–50 cells, $^{**}P < 0.01$. (c) In long-QT hiPSC-CMs, QLQX attenuated abnormal Ca^{2+} transients. $n = 11$ cells, $^{**}P < 0.01$.

function [12]. hiPSC-CMs have also been used to investigate the role of ginsenoside Rb1 in ameliorating aconitine-induced cardiotoxicity by regulating calcium homeostasis [27]. Moreover, three-dimensional engineered heart tissues constructed with 10 normal hiPSC cell-line-differentiated CMs have been used to detect the positive inotropic effects of seven drugs under electrical stimulation [28]. Another study employed human ventricular-like cardiac tissue strips (hvCTS) and organoid chambers (hvCOCs) constructed with disease-free hiPSC-CMs to screen 25 cardioactive chemicals covering different drug categories [29]. Therefore, hiPSC-CMs without disease modeling are suitable for drug evaluation, and we used this model to comprehensively examine the role of QLQX in modulating cardiac Ca^{2+} signals.

We first observed that spontaneous Ca^{2+} transient frequency, an indicator of the beating rate, was remarkably decreased by QLQX in hiPSC-CMs (Figure 2). The hiPSC-CMs used were a mixture of ventricular, atrial, and pacemaker-like cells. hiPSC-CMs also express pacemaker currents (I_f), which are absent in human adult CMs, leading to the innate automaticity of hiPSC-CMs [30]. Therefore, the

spontaneous beating rate of hiPSC-CMs has been widely used to examine the chronotropic effects of drugs. Epidemiological studies have shown that elevated heart rate is a risk factor for cardiovascular disease in healthy people [31]; an increase in heart rate by 10 beats per minute is correlated with a 20% elevated risk of cardiac death [32]. The heart rate-lowering medications can reduce mortality [33]. For example, ivabradine attenuates heart failure by reducing the heart rate of patients [33]. In addition, β-blockers, as commonly prescribed drugs for the treatment of cardiovascular diseases, decrease heart rate to limit myocardial oxygen consumption, thereby improving heart function [34]. Some traditional Chinese medicines, such as ginsenoside, one of the active ingredients of ginseng, reduce heart rate, thereby alleviating cardiac dysfunction and remodeling in heart failure [35].

Ca^{2+} signals reflect the underlying mechanisms of altered cardiac contractile properties during drug treatment. Thus, we comprehensively measured the effect of QLQX on calcium homeostasis in hiPSC-CMs and found that QLQX increased the amplitude of spontaneous Ca^{2+} transients (Figure 2(a) and 2(b)), which was presumably due to its
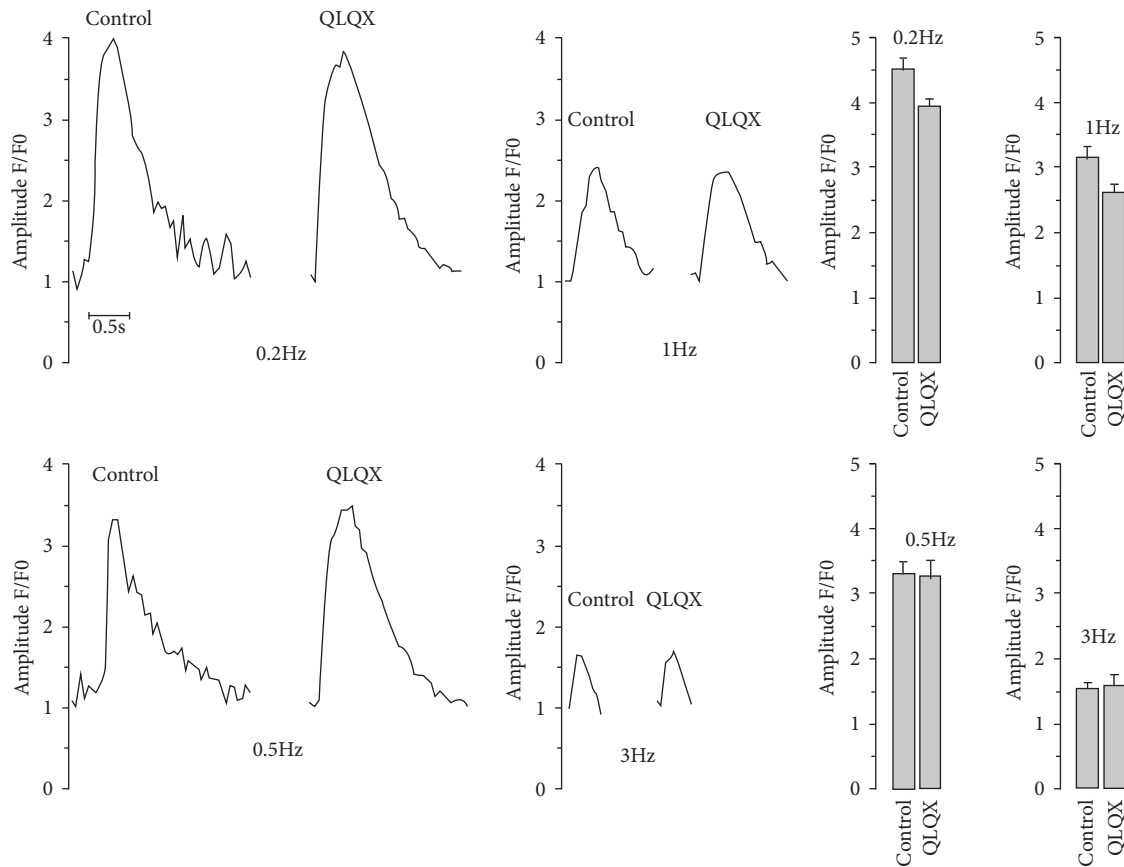
FIGURE 3: Electrical stimulation (0.2–3 Hz)-elicited Ca^{2+} transients were not affected by the QLQX treatment (83 μg/ml, 3 minutes) in hiPSC-CMs. $n = 27$–30 cells.

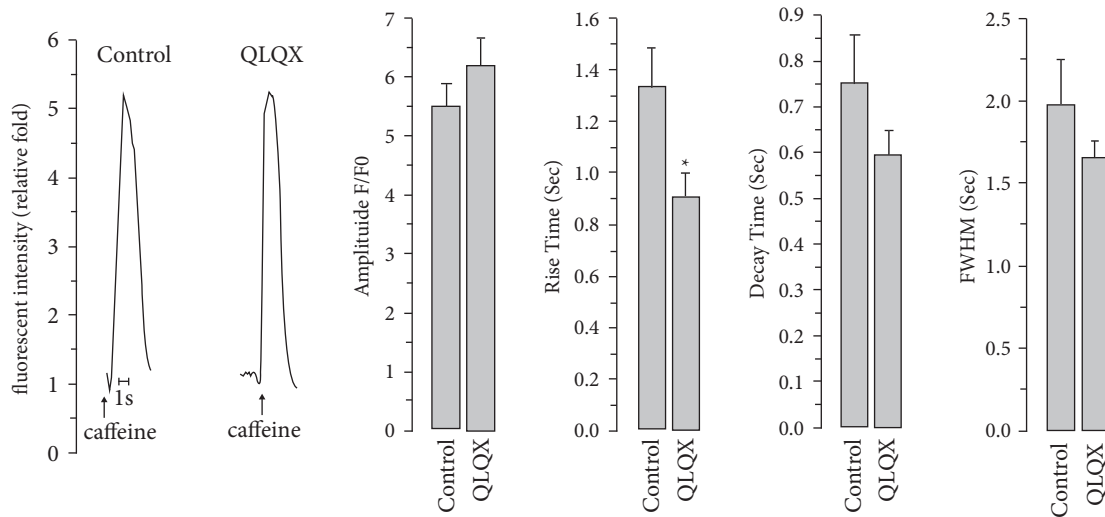
FIGURE 4: QLQX pretreatment (83 μg/ml, minutes) decreased the rise time but not amplitude of caffeine (25 mM)-induced Ca^{2+} rise in hiPSC-CMs. Caffeine application was shown by arrow. $n = 22$–27 cells, $^{*}P < 0.05$.

negative chronotropic effect because hiPSC-CMs harbored a negative force-frequency response (i.e., lowering beating frequency would lead to higher Ca^{2+} transient amplitude). This hypothesis was supported by our observation that QLQX did not change the amplitudes of Ca^{2+} transients elicited by the 0.2–3 Hz electrical stimulation (Figure 3). As

localized calcium signals, calcium sparks reflect the open probability of the RyRs. Indeed, low concentrations of caffeine, an activator of the RyRs, can increase calcium spark frequency [36]. Our results suggest that the QLQX application substantially increased Ca^{2+} spark frequency in both the cytosol and nuclei (Figure 5). Furthermore, the elevated
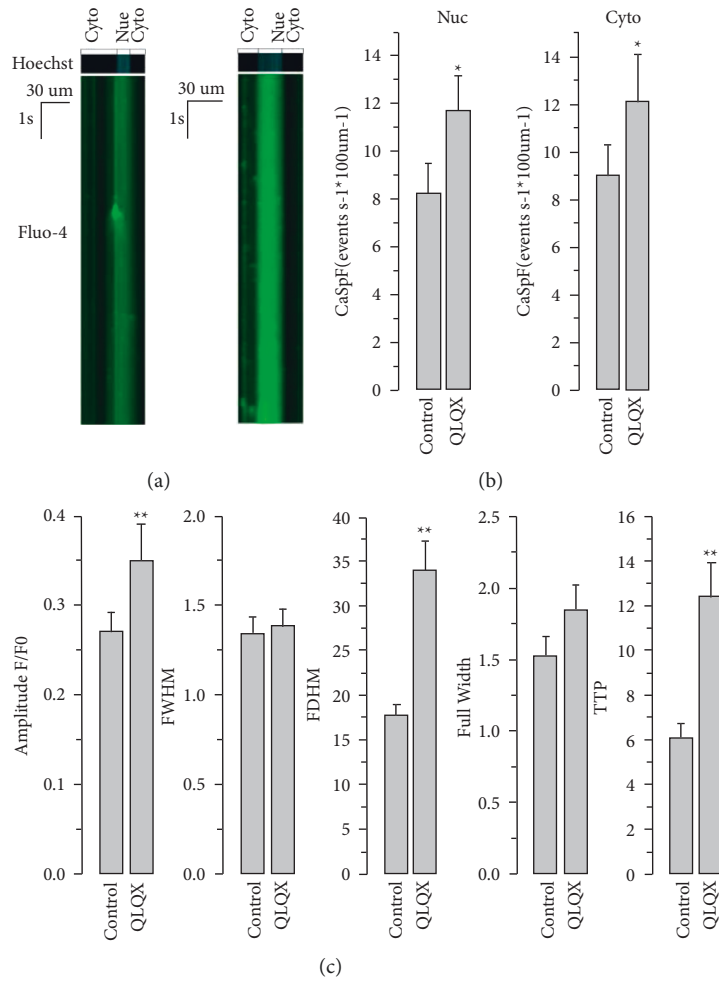
(a)

(b)

(c)

FIGURE 5: QLQX pretreatment (83 μg/ml, 3 minutes) significantly increased Ca^{2+} spark frequency (CaSpF) in both the cytosol and nuclei of hiPSC-CMs (a). Ca^{2+} spark parameters, including full duration at half maximum (FDHM) and time to peak (TTP) were modulated by QLQX (b). $n = 125$ sparks from 20 cells, $^*P < 0.05$, $^{**}P < 0.01$. Nifedipine (3 μM) was used to inhibit spontaneous Ca^{2+} transients.
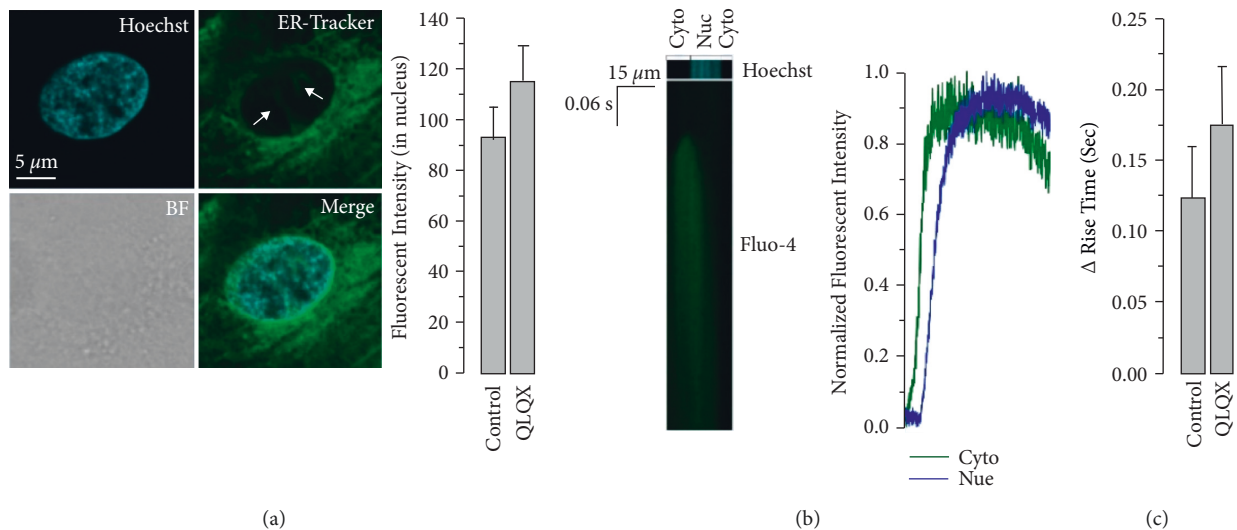

(a)

(b)

(c)

FIGURE 6: QLQX treatment (83 μg/ml, 3 minutes) did not change the nucleoplasmic reticulum (NR) structure in hiPSC-CMs (a). $n = 18–25$ cells. Nuclear Ca^{2+} waves had delayed kinetics compared to that of cytosolic Ca^{2+} waves (b), which was not altered by the QLQX pretreatment in hiPSC-CMs (c). $n = 15–20$ cells. 10 mM external Ca^{2+} was used to promote Ca^{2+} waves.
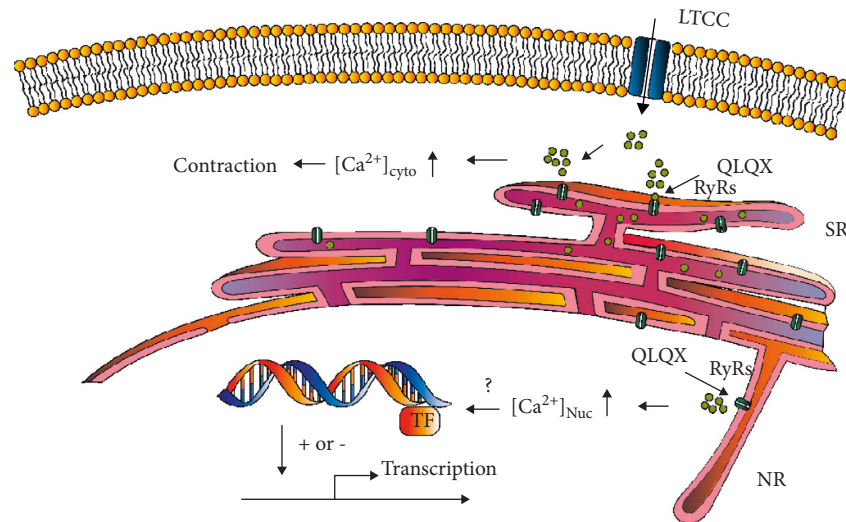
FIGURE 7: Diagram showing that QLQX increased cytosolic/nuclear Ca^{2+} sparks and may potentially modulate nuclear Ca^{2+}-mediated gene transcription.

amplitude and duration of Ca^{2+} sparks after the QLQX treatment indicated stronger Ca^{2+} release. One potential drawback of the increased calcium spark frequency is the elevated level of Ca^{2+} leakage from the SR. However, QLQX did not change the SR Ca^{2+} content of hiPSC-CMs, as determined by the application of a high concentration of caffeine (Figure 4). These results suggest that QLQX may target multiple Ca^{2+}-handling proteins to regulate Ca^{2+} homeostasis in hiPSC-CMs.

In cardiomyocytes, Ca^{2+} exhibits different functions in different subcellular locations. In the cytoplasm, the major function of Ca^{2+} is to regulate cardiac contraction through EC coupling. In the nucleus, Ca^{2+} regulates gene transcription, and the activation of nuclear Ca^{2+}-dependent transcription factors is a part of the process called excitation-transcription coupling (ETC), which is the link between extracellular signals and cardiomyocyte reprogramming [37]. For example, endothelin 1 induces inositol-1,4,5-trisphosphate receptor (IP_3R)-mediated local nuclear Ca^{2+} release, which in turn activates nuclear CaMKII and affects downstream gene transcription during cardiac hypertrophy [38]. Therefore, nuclear Ca^{2+} plays an important role in regulating physiological functions of cardiomyocytes [39]. As mentioned above, our results indicated that QLQX increased the nuclear Ca^{2+} spark frequency; however, the effect of nuclear calcium activation on gene transcription warrants further investigation (Figure 7).

Our HPLC-MS/MS results showed that QLQX contains numerous cardioprotective chemical components. For example, choline can regulate the expression of key calcium-handling proteins, such as STIM1 and Orai1 and attenuate the angiotensin II-induced elevation of intracellular calcium, thereby alleviating the cardiac remodeling induced by abdominal aorta coarctation in rats [40]. As another constituent of QLQX, stachydrine inhibits SR calcium leakage and improves the calcium transient amplitude and cardiac function in mice with transverse aorta constriction [4]. Moreover, caffeic acid regulates calcium and potassium

channels and decreases the heart rate, showing a protective effect on cardiovascular diseases [41]. Therefore, many chemical components of QLQX are closely related to the regulation of cardiac calcium signaling, and the observations in our study may reflect the multitarget pharmacological mechanisms of QLQX.

5. Conclusion

The current study systematically investigated the effect of QLQX on cardiac Ca^{2+} signals using a human cardiomyocyte model, and the results suggest that QLQX could reduce the spontaneous beating frequency of hiPSC-CMs, as reflected by Ca^{2+} transient measurements. Moreover, QLQX substantially promoted the generation of Ca^{2+} sparks in both the cytosol and nucleus. Taken together, these findings provide the first line of evidence that QLQX directly modulates cardiac Ca^{2+} signals in human cardiomyocytes, which may lead to a better understanding of the mechanism of action of QLQX and contribute to the development of novel modern Chinese drugs.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

All authors declare that there are no conflicts of interest.

Authors' Contributions

SL designed the study. YXL performed the experiments, analyzed the data, prepared the figures, and drafted the manuscript. ZZ, XZH, and TTY critically reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgments

Supplementary Materials

Supplementary Table 1: The identified compounds in QLQX by HPLC-MS/MS. (*Supplementary Materials*)

References

[1] M. J. Berridge, M. D. Bootman, and H. L. Roderick, "Calcium signalling: dynamics, homeostasis and remodelling," *Nature Reviews Molecular Cell Biology*, vol. 4, no. 7, pp. 517–529, 2003.

[2] M. Endo, "Calcium ion as a second messenger with special reference to excitation-contraction coupling," *Journal of Pharmacological Sciences*, vol. 100, no. 5, pp. 519–524, 2006.

[3] L. A. Blatter, J. Kockskämper, K. A. Sheehan, A. V. Zima, J. Hüser, and S. L. Lipsius, "Local calcium gradients during excitation-contraction coupling and alternans in atrial myocytes," *The Journal of Physiology*, vol. 546, no. 1, pp. 19–31, 2003.

[4] H. H. Chen, S. N. Wang, T. T. Cao et al., "Stachydrine hydrochloride alleviates pressure overload-induced heart failure and calcium mishandling on mice," *Journal of Ethnopharmacology*, vol. 248, Article ID 112306, 2020.

[5] Y. Yang, Y. Tian, S. Hu et al., "Extract of sheng-mai-san ameliorates myocardial ischemia-induced heart failure by modulating $Ca^{(2+)}$-calcineurin-mediated Drp1 signaling pathways," *International Journal of Molecular Sciences*, vol. 18, no. 9, p. 1825, 2017.

[6] Y. Lu, J. Wu, Y. Sun et al., "Qiliqiangxin prevents right ventricular remodeling by inhibiting apoptosis and improving metabolism reprogramming with pulmonary arterial hypertension," *American Journal of Translational Research*, vol. 12, no. 9, pp. 5655–5669, 2020.

[7] H. Chen, L. Lou, D. Zhang et al., "Qiliqiangxin capsule improves cardiac function and attenuates cardiac remodeling by upregulating miR-133a after myocardial infarction in rats," *Evidence Based Complement Alternative Medicine*, vol. 2019, Article ID 7528214, 9 pages, 2019.

[8] P. Hao, F. Jiang, J. Cheng, L. Ma, Y. Zhang, and Y. Zhao, "Traditional Chinese medicine for cardiovascular disease: evidence and potential mechanisms," *Journal of the American College of Cardiology*, vol. 69, no. 24, pp. 2952–2966, 2017.

[9] X. Li, J. Zhang, J. Huang et al., "A multicenter, randomized, double-blind, parallel-group, placebo-controlled study of the effects of qili qiangxin capsules in patients with chronic heart failure," *Journal of the American College of Cardiology*, vol. 62, no. 12, pp. 1065–1072, 2013.

[10] N. G. Frangogiannis, "Pathophysiology of myocardial infarction," *Comprehensive Physiology*, vol. 5, no. 4, pp. 1841–1875, 2015.

[11] P. Karagiannis, K. Takahashi, M. Saito et al., "Induced pluripotent stem cells and their use in human models of disease and development," *Physiological Reviews*, vol. 99, no. 1, pp. 79–114, 2019.

[12] A. Pointon, A. R. Harmer, I. L. Dale et al., "Assessment of cardiomyocyte contraction in human-induced pluripotent stem cell-derived cardiomyocytes," *Toxicological Sciences*, vol. 144, no. 2, pp. 227–237, 2015.

[13] X. Guan, W. Xu, H. Zhang et al., "Transplantation of human induced pluripotent stem cell-derived cardiomyocytes improves myocardial function and reverses ventricular remodeling in infarcted rat hearts," *Stem Cell Research & Therapy*, vol. 11, no. 1, p. 73, 2020.

[14] S. Li, W. Keung, H. Cheng, and R. A. Li, "Structural and mechanistic bases of nuclear calcium signaling in human pluripotent stem cell-derived ventricular cardiomyocytes," *Stem Cells International*, vol. 2019, Article ID 8765752, 17 pages, 2019.

[15] E. Picht, A. V. Zima, L. A. Blatter, and D. M. Bers, "Sparkmaster: automated calcium spark analysis with ImageJ," *American Journal of Physiology-Cell Physiology*, vol. 293, no. 3, pp. C1073–C1081, 2007.

[16] E. Artimovich, R. K. Jackson, M. B. C. Kilander, Y. C. Lin, and M. W. Nestor, "PeakCaller: an automated graphical interface for the quantification of intracellular calcium obtained by high-content screening," *BMC Neuroscience*, vol. 18, no. 1, p. 72, 2017.

[17] C. I. Spencer, S. Baba, K. Nakamura et al., "Calcium transients closely reflect prolonged action potentials in iPSC models of inherited cardiac arrhythmia," *Stem Cell Reports*, vol. 3, no. 2, pp. 269–281, 2014.

[18] S. S. Lin, C. X. Liu, J. H. Zhang, X. L. Wang, and J. Y. Mao, "Efficacy and safety of oral Chinese patent medicine combined with conventional therapy for heart failure: an overview of systematic reviews," *Evidence-Based Complementary and Alternative Medicine*, vol. 2020, Article ID 8620186, 13 pages, 2020.

[19] L. Guo, H. Yuan, D. Zhang et al., "A multi-center, randomized, double-blind, placebo-parallel controlled trial for the efficacy and safety of shenfuqiangxin pills in the treatment of chronic heart failure (heart-kidney yang deficiency syndrome)," *Medicine*, vol. 99, no. 21, Article ID e20271, 2020.

[20] J. Sun, K. Zhang, W. J. Xiong et al., "Clinical effects of a standardized Chinese herbal remedy, Qili Qiangxin, as an adjuvant treatment in heart failure: systematic review and meta-analysis," *BMC Complementary and Alternative Medicine*, vol. 16, no. 1, p. 201, 2016.

[21] Y. D'Alessandra, M. Chiesa, M. C. Carena et al., "Differential role of circulating microRNAs to track progression and presymptomatic stage of chronic heart failure: a pilot study," *Biomedicines*, vol. 812 pages, 2020.

[22] X. Y. Zhu, S. Q. Wu, S. Y. Guo et al., "A zebrafish heart failure model for assessing therapeutic agents," *Zebrafish*, vol. 15, no. 3, pp. 243–253, 2018.

[23] J. Zhou, Z. Wang, Y. He et al., "Qiliqiangxin reduced cardiomyocytes apoptosis and improved heart function in infarcted heart through pink1/parkin-mediated mitochondrial autophagy," *BMC Complementary Medicine and Therapies*, vol. 20, no. 1, p. 203, 2020.

[24] Y. Wei, X. Liu, L. Hou, W. Che, E. The, and M. V. Jhummon, "Qiliqiangxin affects L type current in the normal and hypertrophied rat heart," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 131830, 4 pages, 2012.

[25] Y. Qin, C. Lv, X. Zhang et al., "Protective effect of Qiliqiangxin against doxorubicin-induced cardiomyopathy by suppressing excessive autophagy and apoptosis," *Cardiovascular Therapeutics*, vol. 2022, Article ID 9926635, 14 pages, 2022.

[26] Y. Lu, M. Xiang, L. Xin et al., "Qiliqiangxin modulates the gut microbiota and NLRP3 inflammasome to protect against

ventricular remodeling in heart failure," *Frontiers in Pharmacology*, vol. 13, Article ID 905424, 2022.

[27] M. Wang, R. Wang, H. Sun, G. Sun, and X. Sun, "Ginsenoside Rb1 ameliorates cardiotoxicity triggered by aconitine via inhibiting calcium overload and pyroptosis," *Phytomedicine*, vol. 83, Article ID 153468, 2021.

[28] I. Mannhardt, U. Saleem, D. Mosqueira et al., "Comparison of 10 control hPSC lines for drug screening in an engineered heart tissue format," *Stem Cell Reports*, vol. 15, no. 4, pp. 983–998, 2020.

[29] W. Keung, P. K. W. Chan, P. C. Backeris et al., "Human cardiac ventricular-like organoid chambers and tissue strips from pluripotent stem cells as a two-tiered assay for inotropic responses," *Clinical Pharmacology & Therapeutics*, vol. 106, no. 2, pp. 402–414, 2019.

[30] P. Garg, V. Garg, R. Shrestha, M. C. Sanguinetti, T. J. Kamp, and J. C. Wu, "Human induced pluripotent stem cell-derived cardiomyocytes as models for cardiac channelopathies: a primer for non-electrophysiologists," *Circulation Research*, vol. 123, no. 2, pp. 224–243, 2018.

[31] R. Xing, A. M. Moerman, R. Y. Ridwan et al., "The effect of the heart rate lowering drug Ivabradine on hemodynamics in atherosclerotic mice," *Scientific Reports*, vol. 8, no. 1, Article ID 14014, 2018.

[32] C. Perret-Guillaume, L. Joly, and A. Benetos, "Heart rate as a risk factor for cardiovascular disease," *Progress in Cardiovascular Diseases*, vol. 52, no. 1, pp. 6–10, 2009.

[33] K. Swedberg, M. Komajda, M. Böhm et al., "Ivabradine and outcomes in chronic heart failure (SHIFT): a randomised placebo-controlled study," *The Lancet*, vol. 376, no. 9744, pp. 875–885, 2010.

[34] G. Koracevic, S. Micic, M. Stojanovic et al., "Compelling indications should be listed for individual beta-blockers (due to diversity), not for the whole class," *Current Vascular Pharmacology*, vol. 19, 2021.

[35] X. Zheng, S. Wang, X. Zou et al., "Ginsenoside Rb1 improves cardiac function and remodeling in heart failure," *Experimental Animals*, vol. 66, no. 3, pp. 217–228, 2017.

[36] L. Borisova, A. Shmygol, S. Wray, and T. Burdyga, "Evidence that a Ca^{2+} sparks/STOCs coupling mechanism is responsible for the inhibitory effect of caffeine on electro-mechanical coupling in Guinea pig ureteric smooth muscle," *Cell Calcium*, vol. 42, no. 3, pp. 303–311, 2007.

[37] S. Ljubojevic and D. M. Bers, "Nuclear calcium in cardiac myocytes," *Journal of Cardiovascular Pharmacology*, vol. 65, no. 3, pp. 211–217, 2015.

[38] X. Wu, T. Zhang, J. Bossuyt et al., "Local InsP3-dependent perinuclear Ca^{2+} signaling in cardiac myocyte excitation-transcription coupling," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 675–682, 2006.

[39] R. R. Resende, L. M. Andrade, A. G. Oliveira, E. S. Guimarães, S. Guatimosim, and M. F. Leite, "Nucleoplasmic calcium signaling and cell proliferation: calcium signaling in the nucleus," *Cell Communication and Signaling*, vol. 11, no. 1, p. 14, 2013.

[40] X. He, S. Yang, J. Deng, Q. Wu, and W. J. Zang, "Amelioration of circadian disruption and calcium-handling protein defects by choline alleviates cardiac remodeling in abdominal aorta coarctation rats," *Laboratory Investigation*, vol. 101, no. 7, pp. 878–896, 2021.

[41] H. Silva and N. M. F. Lopes, "Cardiovascular effects of caffeic acid and its derivatives: a comprehensive review," *Frontiers in Physiology*, vol. 11, Article ID 595516, 2020.