Original Article

Function of the CaMKII–ryanodine receptor signaling pathway in rabbits with left ventricular hypertrophy and triggered ventricular arrhythmia

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BACKGROUND: Calcium calmodulin-dependent kinase II (CaMKII) can be more active in patients with left ventricular hypertrophy (LVH), which in turn causes phosphorylation of ryanodine receptors, resulting in inactivation and the instability of intracellular calcium homeostasis. The present study aimed to determine the effect of CaMKII–ryanodine receptor pathway signaling in rabbits with left ventricular hypertrophy and triggered ventricular arrhythmia.

METHODS: Forty New Zealand rabbits were randomized into four groups (10 per group): sham group, LVH group, KN-93 group (LVH+KN-93), and ryanodine group (LVH+ryanodine). Rabbits in the LVH, KN-93, and ryanodine groups were used to establish a left ventricular hypertrophy model by the coarctation of the abdominal aorta, while those in the sham group did not undergo the coarctation. After eight weeks, action potentials (APs) were recorded simultaneously in the endocardium and epicardium, and a transmural electrocardiogram (ECG) was also recorded in the rabbit left ventricular wedge model. Drugs were administered to the animals in the KN-93 and ryanodine groups, and the frequency of triggered APs and ventricular tachycardia was recorded after the rabbits were given isoprenaline (1 μ mol/L) and high-frequency stimulation.

RESULTS: The frequency (animals/group) of triggered APs was 0/10 in the sham group, 10/10 in the LVH group, 4/10 in the KN-93 group, and 1/10 in the ryanodine group. The frequencies of ventricular tachycardia were 0/10, 9/10, 3/10, and 1/10, respectively. The frequencies of polymorphic ventricular tachycardia or ventricular fibrillation were 0/10, 7/10, 2/10, and 1/10, respectively. The frequencies of triggered ventricular arrhythmias in the KN-93 and ryanodine groups were much lower than those in the LVH group ($P < 0.05$).

CONCLUSIONS: KN-93 and ryanodine can effectively reduce the occurrence of triggered ventricular arrhythmia in rabbits with LVH. The CaMKII–ryanodine signaling pathway can be used as a new means of treating ventricular arrhythmia.

KEY WORDS: CaMKII; Ryanodine receptors; Signaling transduction pathway; Triggered action potential; Ventricular arrhythmia; Left ventricular hypertrophy

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INTRODUCTION

Left ventricular hypertrophy (LVH) is a common pathological occurrence in many cardiovascular diseases. Epidemiological studies have suggested that patients with LVH are more likely to suffer from ventricular cardiac arrhythmia than the general population. The severity of the disease is also much greater in individuals with LVH, and the condition often causes sudden cardiac death. Recent studies have revealed that calcium calmodulin-dependent kinase II (CaMKII) can be more active in patients with LVH, which in turn causes phosphorylation of ryanodine receptors, resulting in inactivation and the instability of intracellular calcium homeostasis. This is an important mechanism contributing to ventricular arrhythmia.

^[1-4] The ventricular wedge is a new tool developed in recent years that can simultaneously record the EKG and action potentials in the endocardium and epicardium. The technique is also advantageous for the study of ventricular arrhythmia. In the present study, we developed an LVH model by coarctation of the abdominal aorta and utilized the left ventricular wedge to observe the occurrence of ventricular arrhythmia in rabbits given isoprenaline. We also investigated the mechanisms of CaMKII inhibitor KN-93 and receptor inhibitor ryanodine.

METHODS

Animals

Forty New Zealand rabbits (2.0-2.5 kg) were obtained from the Experimental Animal Center, Tongji Medical College, Huazhong University of Science and Technology. The rabbits were divided randomly into four groups: sham, LVH, LVH+KN93 (KN-93 group), and LVH+ ryanodine (ryanodine group). Animals in the LVH, KN-93, and ryanodine groups were anesthetized using 20% urethane (1 g/kg). The proximal abdominal aorta was detached surgically, and the aorta above the right kidney was narrowed by 60 % to 70% with a No 4 silk line. Rabbits in the sham group underwent the same surgical procedure, but did not undergo coarctation. The procedure was performed in a sterile environment.^[5] After the surgery, the animals received daily doses of penicillin (800 000 U) for 3 days. All rabbits were fed regularly for 8 weeks.

Ultrasound testing

The rabbits were tested by ultrasound at the beginning of the study and again 8 weeks later. Under

general anesthesia (20% urethane, 1 g/kg), they were placed in a supine position and their chests were shaved. A GE vivid ultrasound system (GE Healthcare) was used, with a 10-second probe, 3.0 to 3.5-cm image depth, a frequency of 11.4 MHz, and a minimized sector scan angle. 2D-slice ultrasound was performed to determine the thickness of the septal wall (SW) and left ventricular posterior wall (LVPW), as well as the left ventricular end-diastolic dimension (LVDd). The weight of the left ventricle was calculated using the Devereux formula^[6]: $1.04 \times [(LVDd + SW + LVPW)^3 - LVDd^3]$

Preparation of rabbit left ventricular wedge model and variables

After 8 weeks, the rabbits were weighed and the left ventricular wedge model was established as previously described.^[7,8] The rabbits in the sham and LVH groups were given Tyrode solution containing isoprenaline (1 μ mol/L). Those in the KN-93 group were given Tyrode solution with KN-93 (1 μ mol/L) for 15 minutes, followed by Tyrode solution with isoprenaline and KN-93 (1 μ mol/L each). Animals in the Ryanodine group were given Tyrode solution containing ryanodine (10 μ mol/L) for 15 minutes, and then given Tyrode solution containing isoprenaline (1 μ mol/L) and ryanodine (10 μ mol/L). After the QT interval stabilized, the action potentials in the endocardium and epicardium and the transmural EKG were recorded. The occurrence of triggered action potentials and ventricular tachycardia (including polymorphic ventricular tachycardia or ventricular fibrillation) was recorded under high-frequency stimulation: S1S1 interval decreased from 200 ms to 120 ms; pulse length 10 ms, 20 stimulations per set, 10-s cycle length, 5 repeats; stimulation at 2 $\times$ threshold voltage, 1-ms bandwidth. The weight of the heart and the left ventricle were measured after the experiment.

Observations

The following observations were made: a) QT interval – the distance between the signal starting point and the intersection between t-wave and the origin; b) triggered action potential – the after-depolarization triggered when complete repolarization was achieved; c) ventricular tachycardia – the reproducible occurrence of five or more triggered action potentials after the stimulation was stopped; d) polymorphic ventricular tachycardia – the appearance of polymorphic ventricular tachycardia or ventricular fibrillation after

the appearance of ventricular tachycardia.

Testing agent and solutions

KN-93 and DMSO were purchased from Sigma. Ryanodine was purchased from Enzo Life Sciences. The other agents were of analytical grade. Regular Tyrode solution contained (mmol/L) KCl 4, NaCl 129, NaH_2PO_4 0.9, NaHCO_3 20, CaCl_2 1.8, MgSO_4 0.5, glucose 5.5, at pH 7.4 and saturated with 95% O_2 / 5% CO_2 . High-potassium Tyrode solution contained (mmol/L) KCl 24, NaCl 109, NaH_2PO_4 0.9, NaHCO_3 20, CaCl_2 1.8, MgSO_4 0.5, glucose 5.5, at pH 7.4 and saturated with 95% O_2 / 5% CO_2 . Isoprenaline was dissolved in Tyrode solution at a concentration of 1 $\mu\text{mol/L}$. KN-93 (1 $\mu\text{mol/L}$) and ryanodine (10 $\mu\text{mol/L}$) were dissolved first in DMSO, followed by Tyrode solution.

Statistical analysis

The data were analyzed using SPSS16.0. All quantities were expressed as mean $\pm$ standard deviation. All statistics underwent the paired-t test and Fisher's exact test. Values of $P < 0.05$ were deemed statistically significant.

RESULTS

Pathological changes

Compared to the sham group, the hearts of the rabbits in the other groups were significantly enlarged (Table 1). Left ventricular weight, the ratio of left ventricle weight to the rabbit's body weight, and the left

ventricular free wall thickness were also significantly larger than those of the sham group. These data indicate that the model was established successfully.

Ultrasonography

The hearts of rabbits in the LVH, KN-93, and ryanodine groups had significantly thicker left ventricular walls than those of the rabbits in the sham group (Table 2). The left ventricular weights of hearts from these groups were also significantly higher than those from the sham group.

Ventricle wedge and stimulation

Animals in the sham group exhibited no triggered action potentials or episodes of ventricular arrhythmia (Table 3). In the LVH group, 9 out of the 10 animals exhibited triggered action potentials (Figure 1), and 9 of the 10 animals exhibited ventricular arrhythmia

Table 3. Comparison of triggered action potentials and cardiac arrhythmia due to programmed stimulation among various groups

Groups	Triggered AP ^a	V-Tach ^b	PMVT ^c or V-Fib ^d
Sham (n=10)	0	0	0
LVH (n=10)	10	9	7
KN-93 (n=10)	4	3	2
Ryanodine (n=10)	1	1	1

^a: action potential; ^b: ventricular tachycardia; ^c: polymorphic ventricular tachycardia; ^d: ventricular fibrillation.

Figure 1. Action potential in the epicardium and endocardium and transmural EKG were simultaneously recorded. Triggered action potential was observed after high-frequency programmed stimulation.

Table 1. Comparison of cardiac anatomical parameters among different groups (mean $\pm$ SD, n = 10)

Groups	BW (kg)	LVM (g)	LVM/BW	LVFW (mm)
Sham	2.10 $\pm$ 0.29	3.26 $\pm$ 0.27	1.56 $\pm$ 0.10	4.5 $\pm$ 0.2
LVH	2.51 $\pm$ 0.31	6.94 $\pm$ 0.59*	2.78 $\pm$ 0.14*	6.6 $\pm$ 0.3*
KN-93	2.49 $\pm$ 0.32	6.90 $\pm$ 0.58*	2.79 $\pm$ 0.18*	6.5 $\pm$ 0.2*
Ryanodine	2.52 $\pm$ 0.30	6.96 $\pm$ 0.56*	2.78 $\pm$ 0.17*	6.4 $\pm$ 0.3*

BW: body weight; LVM: left ventricle mass; LVM/BW: ratio of BW and LVM; LVFW: left ventricle free wall; all groups were compared with the sham group, * $P < 0.05$.

Table 2. Comparison of morphological and structural parameters of the heart for different groups of rabbits by ultrasound (mean $\pm$ SD, n = 10)

Groups	SW (mm)	LVPW (mm)	LVDd (mm)	LVM (g)	LVM/BW
Sham	3.04 $\pm$ 0.23	3.03 $\pm$ 0.28	10.90 $\pm$ 0.64	3.76 $\pm$ 0.55	1.79 $\pm$ 0.12
LVH	4.18 $\pm$ 0.24*	4.06 $\pm$ 0.23*	11.08 $\pm$ 0.60	6.11 $\pm$ 0.69*	2.44 $\pm$ 0.10*
KN-93	4.21 $\pm$ 0.26*	4.08 $\pm$ 0.22*	11.11 $\pm$ 0.63	6.17 $\pm$ 0.50*	2.49 $\pm$ 0.12*
Ryanodine	4.23 $\pm$ 0.30*	4.09 $\pm$ 0.25*	11.02 $\pm$ 0.55	6.15 $\pm$ 0.64*	2.45 $\pm$ 0.12*

SW: septal width; LVPW: left ventricle posterior wall; LVDd: left ventricular end-diastolic dimension; LVM: left ventricle mass; LVM/BW: ratio between LVM and BW; all groups were compared with the sham group, * $P < 0.05$.

(Figure 2). Polymorphic ventricular tachycardia and ventricular fibrillation occurred in 7 of the 10 animals tested (Figures 3, 4). In the KN-93 group, triggered action potentials occurred in 4 of the 10 rabbits, ventricular tachycardia appeared in 3 of the 10 rabbits, and ventricular tachycardia and ventricular fibrillation were present in only 2 of the 10 rabbits. These numbers were significantly different from those of the LVH group ($P<0.05$). The ryanodine group exhibited even fewer

Figure 2. Action potential in the epicardium and endocardium and transmurally EKG were simultaneously recorded. Ventricular tachycardia was observed after high-frequency programmed stimulation.

Figure 3. Action potential in the epicardium and endocardium and transmurally EKG were simultaneously recorded. Polymorphic ventricular tachycardia was observed after high-frequency programmed stimulation.

Figure 4. Action potential in the epicardium and endocardium and transmurally EKG were simultaneously recorded. Polymorphic ventricular fibrillation was observed after high-frequency programmed stimulation.

cardiac abnormalities: triggered action potentials, 1/10; ventricular tachycardia, 1/10; and ventricular tachycardia and ventricular fibrillation, 1/10. These results were significantly different from those of the LVH group ($P<0.05$). The results indicated that both KN-93 and ryanodine can be used to reduce the occurrence of LVH.

DISCUSSION

Ventricular cardiac arrhythmia, especially ventricular tachycardia and ventricular fibrillation, is a common cause of sudden cardiac death for patients with LVH.^[9-12] Recent studies have revealed that derangement in calcium homeostasis contributes to the arrhythmia.^[13-16] Calcium leakage during ventricular dilation and calcium overload in the cytoplasm may trigger ventricular arrhythmia by generating a transient inward current (ITI) via the sodium calcium exchanger (NCX) and delayed after depolarization (DAD). Such a condition can be triggered more easily under acute catecholamine stimulation, which can occur during exercise or when stimulated.^[17-19] The present study used isoprenaline, the ventricular wedge, and high-frequency stimulation to reveal that LVH may cause a higher incidence of triggered action potentials and ventricular arrhythmia. Polymorphic ventricular tachycardia or ventricular fibrillation can also occur often.

Currently, medicines treating cardiac arrhythmia work mainly on ion-channel receptors on the cell membrane. These medications are usually not effective, which is why we are driven to find new treatments. More recent studies have focused on the molecular signals within the cell, such as calcium homeostasis, in order to determine the cause and identify new treatments for cardiac arrhythmia.^[20,21] The present study has revealed that CaMKII inhibitor KN-93 and inhibitor ryanodine can reduce the occurrence of LVH-triggered action potentials and ventricular arrhythmias. This means that the CaMKII-ryanodine receptor signaling pathway is very important in LVH-induced ventricular arrhythmia. A recent study has also revealed that the expression and function of CaMKII can increase with LVH.^[13,22-24] This causes over-phosphorylation of ryanodine receptors, leading to abnormalities in the release of calcium into the cytoplasm, which consequently facilitates the occurrence of DAD and even ventricular arrhythmia.

Ryanodine is a key protein determining cellular calcium levels, and any malfunction can disrupt

calcium homeostasis.^[25-28] The present study revealed that compared with KN-93, ryanodine can more effectively inhibit arrhythmia, indicating that ryanodine receptors play a central role in LVH-induced ventricular arrhythmia. Studies also showed that two hereditary diseases, catecholaminergic polymorphic ventricular tachycardia and arrhythmogenic right ventricular cardiomyopathy, are both related to mutations in ryanodine receptors.^[29-31] Ryanodine receptors are controlled by CaMKII- and PKA-mediated phosphorylation,^[32,33] which explains why ryanodine has a greater inhibitory effect on arrhythmic activity. While KN-93 has shown an inhibitory effect on CaMKII, it cannot control PKA. Therefore, KN-93 is a much weaker inhibitor of cardiac arrhythmia than ryanodine.

The prevention of cardiac arrhythmia and sudden cardiac death has been a clinical challenge. Studies have focused on cellular signaling pathways as a new focus. The findings of the present study suggest that the CaMKII-ryanodine pathway can be an effective therapeutic target for cardiac arrhythmia, and that drugs effectively targeting the pathway may be powerful in treating cardiac arrhythmia in the future.

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