Original Article
Verapamil suppresses cardiac alternans and ventricular arrhythmias in acute myocardial ischemia via ryanodine receptor inhibition

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Abstract: T-wave alternans (TWA) is a potent arrhythmia substrate under the conditions of acute myocardial ischemia. Abnormal intracellular calcium cycling contributes to the genesis of cardiac alternans. Ryanodine receptor (RyR) is a pivotal Ca2+ cycling protein central to Ca2+ signaling in the heart. Here, we investigated the potential role of RyR in cardiac alternans and ventricular arrhythmias in acute myocardial ischemia. Transmembrane action potentials were simultaneously recorded from epicardium and endocardium together with a transmural ECG and isometric contraction force in the arterially perfused left ventricular wedge preparations. Calcium alternans were induced by incremental frequency of field stimulation in rat ventricular myocytes. TWA, mechanical alternans and ventricular arrhythmias were reproducibly induced by rapid pacing in the acute ischemic wedge preparations. Compared with control group, calcium alternans ratio and spontaneous calcium release were increased in acute ischemic myocytes. Verapamil, a phenylalkylamine calcium channel blocker, can successfully abolish spontaneous calcium release, TWA, and ventricular arrhythmias. The inhibition effect of verapamil could be diminished by low concentration of ryanodine (10 nmol/L). However, nifedipine, a dihydropyridine calcium channel blocker, could not block TWA or arrhythmias. Moreover, verapamil, but not nifedipine, significantly decreased ROS production in ischemic myocytes. Collectively, our results indicate that verapamil can significantly inhibit the development of cardiac alternans and ventricular arrhythmias in acute myocardial ischemia, and the mechanism was related to the inhibition of RyR and the protective function to oxidative stress.

Keywords: Verapamil, cardiac alternans, arrhythmias, myocardial ischemia, ryanodine receptor

Introduction

T wave alternans (TWA), defined as periodic beat-to-beat variation in the amplitude or shape of the T wave in an electrocardiogram, has long been observed in different settings including acute myocardial ischemia (myocardial infarction, unstable angina, coronary artery spasm) [1-3]. TWA has been found to precede the onset of life-threatening ventricular arrhythmias, and may serve as an important prognostic indicator of ventricular tachycardia and sudden cardiac death in acute myocardial ischemia [2-4].

TWA is the manifestation of beat-to-beat alternation in the action potential duration (APD) and intracellular calcium transient [5, 6]. Alternation of intracellular calcium cycling between the plasma lemma and sarcoplasmic reticulum (SR) leads to corresponding changes in action potential morphology and alternated contraction force mechanistically via sarcolemmal calcium cycling ion channels [7, 8]. Regulation of Ca2+ release from the SR via ryanodine receptor (RyR), has been recognized as a key factor in the genesis of electromechanical alternans [9, 10].

Altered redox balance and redox-mediated changes in Ca2+ handling have been proved to be important pathogenic factors in different cardiac diseases, including ischemic heart dis-
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Figure 2. There were no obvious alterations of action potential duration in epicardium (Epi), endocardium (Endo), T wave or isometric contractile force (ICF) in a normal rabbit left ventricular wedge preparation paced at 5 Hz stimulation.

Measurements of ROS production

Changes in ROS production were measured with the fluorescent indicator 5-(and-6) chloromethyl-20, 7-dichlorodihydrofluoresceindiacetate (DCFDA, 10 µM, Molecular Probes). After subtraction of background fluorescence, the signal (DF) was normalized to maximum fluorescence attained by application of 10 mM \( \text{H}_2\text{O}_2 \) (FMAX). Cells were incubated in KRH solution with verapamil (1.5 µM), nifedipine (1.5 µM), or mercaptopropionyl glycine (750 µM) for one hour. Myocytes were allowed to settle onto the coverslip at the bottom of the custom made perfusion chamber and continuously perfused with normal KRH solution or ischemia injury solution or drugs mentioned above for 20 min. The fluorescent intensity was measured every 4 seconds, after experiment the initial and ultimate value were compared.

Statistical analysis

Data are presented as mean ± SEM unless otherwise indicated. Statistical analysis of the data was performed using Student t-test for paired data or 1-way ANOVA coupled with Scheffé’s test as appropriate. Fisher’s exact test was used as appropriate for non-parametric data [25]. Significance was defined as a value of \( P \)-values < 0.05.

Results

TWA, APD alternans and TDR in acute myocardial ischemia wedge preparations

During rapid pacing (cycle length = 200 ms), there were no noticeable alternans of APD or T
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Wave in control group (Figure 2). The alteration of APD in epicardium in every other two beats, together with the development of TWA in acute ischemic wedge preparations are shown in Figure 3. A more marked beat-to-beat change in epicardial APD than endocardial APD resulted in an alternated change in TDR (Figure 6B). Discordant T Wave and mechanical alternans (a beat with larger T wave was associated with weaker ICF) occurred in all the wedges (n = 10) in acute myocardial ischemia group. Ventricular arrhythmias were reproducibly induced in 5 wedges in acute myocardial ischemia group (Figure 4).

Figure 3. The alteration of action potential duration in epicardium in every other two beats, together with the development of discordant T Wave and mechanical alternans in acute ischemic wedge preparations. ICF: isometric contractile force; Epi: epicardium; Endo: endocardium.

Figure 4. Ventricular arrhythmia developed after the cessation of rapid ventricular pacing by which TWA was induced steadily in acute ischemic wedge preparations. Epi: epicardium; Endo: endocardium.

Figure 5. Verapamil (1 μM) inhibited the development of cardiac alternans and ventricular arrhythmias in acute ischemic wedge preparations. ICF: isometric contractile force; Epi: epicardium; Endo: endocardium.

Calcium alternans in acute ischemic myocytes

Calcium alternans ratio of each group was shown in Figure 8. Compared with control group, calcium alternans ratio and SCR
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Figure 6. Beat-to-beat changes in action potential duration (APD) and transmural dispersion of repolarization (TDR) in wedge preparations at 5 Hz stimulation. A: There were no noticeable alternans of APD or TDR in control group. B: Beat-to-beat changes in APD were more marked in the epicardium (Epi) than the endocardium (Endo) in acute myocardial ischemia wedges. C: Verapamil at 1 μM abolished beat-to-beat changes in APD and TDR in acute myocardial ischemia wedges. D: Nifedipine (1 μM) could not block alternans of APD or TDR. E: Low concentration of ryanodine (10 nM) diminished the inhibition effect on alternans of APD and TDR by verapamil. F: High concentration of ryanodine (3 μM) enhanced the inhibition effect on alternans of APD and TDR by nifedipine. Nif: nifedipine; Ry: ryanodine; Ver: verapamil.

Figure 7. The occurrence of T wave alternans (TWA) and ventricular tachycardia/fibrillation (VT/VF) in each group. Isc: ischemic group; MPG: mercaptopropionyl glycine; Nif: nifedipine; Ry: ryanodine; Ver: verapamil.

increased significantly in the ischemia group. Verapamil (1 μM) significantly decreased calcium alternans ratio and SCR in acute ischemic myocytes. Neither nifedipine nor ryanodine decreased calcium alternans ratio and SCR.
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Effect of verapamil on ROS production in acute ischemic myocytes

Compared with controls (2.9 ± 0.3 F/F₀, n = 22), ROS production increased approximately 1.2-fold in ischemic myocytes (3.5 ± 0.3 F/F₀, n = 18, Figure 9). MPG (2.7 ± 0.5 F/F₀, n = 20) and verapamil (3.1 ± 0.5 F/F₀, n = 28) significantly decreased ROS levels in ischemic myocytes.

Discussion

In the present study, using arterially-perfused rabbit left ventricular wedge, the ECG, APD and ICF were simultaneous recorded. The results of this study indicated that action potential alternans were more likely originating from epicardium than endocardium, accompanying with TWA, mechanical alternans, and ventricular arrhythmias.

In the clinical setting, the optimal detection of TWA requires a heart rate in the range 90-110 bpm, which may be achieved by exercise testing, pharmacological intervention, or rapid atrial pacing [3, 23]. The present study showed that TWA and ventricular arrhythmias were reproducibly induced by rapid pacing (cycle length = 200 ms) in the acute ischemic wedge preparations. It is a reliable model to study the mechanism underlying TWA and ventricular arrhythmias in ischemic condition.

TWA, APD alternans and mechanical alternans in acute myocardial ischemia

Previous studies showed that action potential alternans generated TWA in cellular level [26, 27]. Selvaraj reported that a greater number of epicardial sites exhibited alternans than endocardial sites at cycle length 600 ms in patients with cardiomyopathy [28]. In the present study, a significant beat-to-beat change in epicardial APD created beat-to-beat alteration in transmural voltage gradient that manifested as TWA on the ECG. A more noteworthy beat-to-beat change in epicardial APD than endocardial APD resulted in an alternated change in TDR (Figure 6B). Interestingly, contraction force alternated in an opposite phase (“out of phase”) with APD and T-wave. Larger epicardial APD and greater T wave was associated with smaller contraction force.

Electromechanical cardiac alternans has been closely associated with susceptibility to ventricular arrhythmias in acute myocardial ischemia.
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TWA has been shown to be an effective tool for identifying high-risk patients in the presence of myocardial ischemia. The risk of cardiac arrhythmia in TWA positive patients is close to 4 times than in TWA negative [29]. Repolarization alternans at the level of the single cell could trigger ventricular fibrillation [30]. Previous studies showed that TWA could lead to reentry and triggered activity, resulting in the genesis of ventricular fibrillation [31-33]. In the present study, 5 of 10 acute myocardial ischemia wedges developed ventricular tachycardia and/or fibrillation after TWA, which was consistent with clinical manifestations and preceded experiments.

Verapamil and RyR in intracellular calcium transient in acute myocardial ischemia

Recent study has shown that intracellular Ca^{2+} oscillation plays a critical role in the genesis of APD alternans and TWA [34]. Previous studies indicated that RyRs are critical in excitation contraction coupling [34, 35]. Ryanodine activates the open probability of RyR at low concentration (~10 nM) while has inhibitory effects at high concentration (~10 μM) [36].

Our study proved that verapamil (1 μM), a phenylalkylamine L_{\text{CaL}} blocker, might have blocking effect on RyR and electromechanical alternans by improving discordant repolarization alternans and abnormal intracellular calcium homeostasis. Nifedipine (1 μM), a dihydropyridine calcium channel blocker, had no effect on RyR and electromechanical alternans. Inhibition of RyR (3 μM ryanodine) enhanced the antiarrhythmic effect of nifedipine. Moreover, enhance of RyR function (10 nM ryanodine) reduced the antiarrhythmic effect of verapamil. The nature of the beat-to-beat response of T wave and contraction may depend on the activity of the LTCC and SR function.

Intracellular calcium overload and spontaneous calcium wave of SR could induce arrhythmias in ischemic heart disease [37]. Previous clinical trials showed that verapamil had a positive influence on main end-point events in patients with myocardial infarction [19, 20]. Verapamil has been reported to effectively suppress ventricular arrhythmias in rats subjected to myocardial ischemia [38]. Apart from L-type calcium channel blockade, it also occupies functions such as reducing sarcoplasmic reticulum calcium content [34], protecting energy metabolism, maintaining mitochondrion function [13], stabilizing connexin 43 at the gap junction and promoting electrophysiological property [38]. In vitro studies have suggested that verapamil may bind to RyR2 and inhibit its channel activity [39].

Oxidative stress, redox modification of RyR and cardiac alternans in acute myocardial ischemia

Cardiac alternans is highly dependent on SR Ca^{2+} regulatory proteins and their redox status [40-42]. Sulfhydryl oxidation of reactive cyste-
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RyR molecules could increase RyR open time probability, thus producing ‘leaky’ RyR channels [43, 44]. Previous studies showed that ROS production and oxidative stress increased in ischemic cardiac diseases [45-51]. Superoxide generation occurred during ischemia from the ubisemiquinone site of the mitochondrial electron transport chain [52-54]. The present study showed that ROS production increased approximately 1.2 fold in ischemic myocytes. We also found that verapamil could decrease ROS level and cardiac alternans. MPG, a powerful anti-oxidant with the ability to reduce ROS, could not suppress TWA or arrhythmias. These results addressed the significance of oxidative/anti-oxidative balance in ischemic cardiac disease.

Clinical implications

Acute myocardial ischemia and associated electromechanical instability predispose to malignant ventricular arrhythmias, leading to sudden cardiac death. Several interesting electrocardiographic and mechanical manifestations observed in the present study may enhance our understanding of arrhythmogenic markers in acute myocardial ischemia.

Verapamil inhibits cardiac alternans with multiple role including L-type calcium channel blockade, RyR inhibition, and anti-oxidative effects, thus making it a prospective drug for ischemic ventricular arrhythmia.

Study limitations

Although our present study and other previous studies have demonstrated that increased $I_{Ca-L}$ can cause abnormal fluctuating of intracellular Ca$^{2+}$ and cardiac alternans in acute myocardial ischemia. The exact linking mechanism of the beat-to-beat electromechanical abnormalities in acute myocardial ischemia is unclear. Remolding of $I_{Ca}$, $I_k$ and $I_{Na/ Ca}$ may also be involved in the beat-to-beat cardiac alternans.

Conclusions

In summary, our study shows that verapamil inhibits discordant cardiac alternans and ventricular arrhythmias via the suppression of RyR and ROS in acute myocardial ischemia. Our data suggests that SR function may contribute importantly to cardiac alternans and arrhythmias in acute myocardial ischemia.

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Disclosure of conflict of interest

None.

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