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Piceatannol facilitates conduction block and ventricular fibrillation induction in ischemia-reperfused rabbit hearts with pacing-induced heart failure ☆,☆☆

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ABSTRACT

Background: Piceatannol, a hydroxystilbene natural product, has been reported to exert antiarrhythmic action via I_{Na} inhibition and slow I_{Na} inactivation in ischemia-reperfused (IR) rat hearts. The present study aimed to clarify the proarrhythmic property of piceatannol during regional IR injury in failing rabbit hearts.
Methods: Heart failure (HF) was induced by rapid right ventricular pacing for 4 weeks. The IR model was created by coronary artery ligation for 30 min, followed by reperfusion for 15 min in vivo. Simultaneous voltage and intracellular Ca^{2+} (Ca_i) optical mapping was then performed in isolated Langendorff-perfused hearts ($n = 11$ in each HF and control group). Action potential duration (APD) restitution, arrhythmogenic alternans and VF inducibility were evaluated by a dynamic pacing protocol. Conduction velocity was measured along lines across the IR and non-IR zones during pacing. Piceatannol (10 μM) was administered after baseline studies.
Results: In the HF group, piceatannol decreased conduction velocity, induced rate-dependent regional inhomogeneity of conduction delay and wavelength shortening, slowed Ca_i decay, and facilitated arrhythmogenic alternans instead of APD prolongation to increase VF inducibility. In the control group, the proarrhythmic effects of piceatannol on APD restitution, arrhythmogenic alternans and conduction delay were offset by its antiarrhythmic effects (APD and wavelength prolongation), resulting in a neutral effect on VF inducibility.
Conclusions: Piceatannol (10 μM) is proarrhythmic in failing rabbit hearts with regional IR injury. The increased VF inducibility by piceatannol in HF suggests that its undesirable effects are more pronounced than its benefits in failing hearts.

1. Introduction

Piceatannol (3,3′,4′,5-tetrahydroxystilbene, astringinin), a hydroxystilbene natural product, has been reported to be antiarrhythmic against ischemia–reperfusion (IR) injury via slowing sodium channel (I_{Na}) inactivation to increase the effective refractory period (ERP) in association with decreasing I_{Na} to stabilize the membrane excitability in rat hearts [1]. However, the cardiac safety of piceatannol yet remains to be established since a decrease of the maximal rate of upstroke of action potential via decreasing I_{Na} also results in conduction delay, which may contribute to the genesis of arrhythmias [2]. Venkataraman et al. reported that reperfusion corrects intracellular Ca^{2+} (Ca_i) handling to normal, but tissue conduction velocity (CV) remains slowed in isolated mouse hearts with IR injury [3]. In a coverslip IR model, IR arrhythmias were associated with delayed recovery of CV relative to recovery of excitability [4]. Therefore, it is possible that piceatannol further aggravates regional inhomogeneity in CV and produces obstacles for reentrant wavefront formation during regional IR injury [5]. I_{Na} dysfunction was also observed in failing hearts [6,7], and CV was found to significantly decrease in failing hearts by 6–20% [8]. The slower CV in failing hearts was reported to be more pronounced during ischemia, which may account for a higher propensity for reentrant arrhythmias during acute regional ischemia [9]. It is possible that preexisting conduction delay in failing hearts also facilitates reentrant arrhythmia during regional IR injury. For these reasons, the synergistic effects on CV slowing from IR injury and heart failure (HF) may amplify the arrhythmogenicity of piceatannol to overwhelm its antiarrhythmic effects. In this study, we performed simultaneous membrane potential (V_m) and Ca_i mapping in Langendorff-

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perfused rabbit hearts to test the hypotheses that preexisting HF intensifies impulse propagation disturbance caused by IR injury, and that piceatannol further aggravates anisotropic conduction to facilitate ventricular fibrillation (VF) induction in failing hearts with regional IR injury.

2. Methods

The research protocol was approved by the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital (approval No. 2011121301) and conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health. Twenty-two adult New Zealand white rabbits (2.8–3.6 kg) were used, including eleven in each of the HF and control groups.

2.1. Pacing-induced HF

The rabbits were premedicated with intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg), intubated and anesthetized with isoflurane. When the rabbits were fully anesthetized and unresponsive to physical stimuli, the chests were opened via a right lateral thoracotomy. An epicardial pacing lead was placed in the lateral wall of the right ventricle and connected to an Itrel III pacemaker (Medtronic, Inc., Minneapolis, MN, USA) for tachycardia pacing. After 1 week of convalescence, the rabbit ventricle was paced at 312 bpm for 4 weeks. The rabbits were monitored by clinical examination and echocardiography to observe the progressive process of developing HF. When the rabbits became lethargic, showed loss of appetite, developed respiratory distress, and when a significant decrease in left ventricular (LV) ejection fraction was documented by echocardiography, a secondary surgery was performed for optical mapping studies.

2.2. IR model creation

Regional ischemia was induced by a method used in our previous study [10]. Briefly, the rabbits were premedicated with intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg), intubated and anesthetized with isoflurane. When the rabbits were fully anesthetized and unresponsive to physical stimuli, the chests were opened via a left thoracotomy at the fifth intercostal space, and ribs were retracted to expose the heart. The obtuse marginal branch of the left circumflex artery was identified and ligated halfway between the atrioventricular groove and the cardiac apex for 30 min [11], followed by reperfusion. Occlusion of the obtuse marginal branch resulted in darker color of the epicardium in the ischemic zone relative to the intact tissue in all animals. The limb lead electrocardiogram (ECG) was monitored continuously.

2.3. Optical mapping of isolated rabbit hearts

After reperfusion for 15 min, the hearts were quickly excised and Langendorff-perfused with 37 °C Tyrode's solution (composition in mmol/L: NaCl 125, KCl 4.5, $MgCl_2$ 0.25, $NaHCO_3$ 24, NaH_2PO_4 1.8, $CaCl_2$ 1.8, glucose 5.5, and albumin 50 mg/L in deionized water), equilibrated with 95% O_2 and 5% CO_2 to maintain a pH of 7.4. The coronary perfusion pressure was regulated and maintained at 70–80 cmH_2O. A Ca^{2+}-sensitive dye (0.5 mg rhod-2 AM. Molecular Probes, Eugene, OR, USA) and a voltage-sensitive dye (RH237, Molecular Probes) were administered. The hearts were illuminated using a laser (Millennia, Spectra-Physics Inc., Santa Clara, CA, USA) at a wavelength of 532 nm. The emitted fluorescence was filtered and acquired simultaneously with two charge-coupled device cameras (CA-D1-0128T; Dalsa Inc., Billerica, MA, USA) at 269 frames/s. Digital images (128 × 128 pixels) were gathered from the epicardium of the LV (25 × 25 mm^2 area), resulting in a spatial resolution of 0.2 × 0.2 mm^2 per pixel. Motion artifacts were suppressed by 5 μM cytochalasin D. The average fluorescence level ($\overline{F}$) of an individual pixel was first calculated for the duration of recording. The ratio on each pixel was than calculated as $(F - \overline{F}) / \overline{F}$, color-coded with shades of red (depolarization) or blue (repolarization), and animated to show propagation patterns in the mapping field.

2.4. Experimental protocols

A bipolar catheter was inserted into the right ventricular apex for pacing at twice the threshold. The ERP was measured by giving a premature stimulus after 8 beats at a 400 ms pacing cycle length (PCL). The action potential duration (APD_{80}) (APD interval between the phase 0 and the time of 80% repolarization) restitution curve was determined, and APD_{80} and Ca_i alternans were induced by a dynamic pacing protocol: decremental right ventricular pacing starting at a constant PCL of 300 ms, after which PCL was initially decreased in 20-ms steps and 10-ms steps after PCL of 200 ms, until loss of 1:1 capture or induction of arrhythmias was observed. The VF inducibility was defined as the ability to provoke sustained VF (>2 min) by the dynamic pacing protocol [12]. Defibrillation using epicardial patch electrodes was performed for sustained VF. Piceatannol (10 μM) was administered for 12 min, and then the pacing protocols were repeated following the baseline studies.

2.5. Data analysis

APD_{80} restitution curves were constructed at selected evenly-spaced sites. The maximum slope of APD_{80} restitution curve was determined after curve fitting by first-order exponential fitting with Origin software (Micro-Cal, Northampton, MA, USA). We used monoexponential fitting to compute the time constant (τ) of the decay portion of the Ca^{2+} transient [13] at a PCL of 400 ms. Alternans was measured by the differences in the APD_{80} (ΔAPD_{80}) and Ca_i amplitudes between 2 consecutive beats. The phase was considered positive for a short–long APD and a small–large Ca_i amplitude sequence (color-coded by red), and was negative for a long–short APD and a large–small Ca_i amplitude sequence (color-coded by green). Spatially discordant alternans (SDA) was evidenced by the presence of both red and green regions separated by a nodal line. The APD_{80} alternans threshold was defined as the longest PCL required to produce an absolute $\Delta APD_{80} > 8$ ms. The Ca_i alternans ratio (ΔCa_i peak) was defined as $1 - (B / A)$, where B and A were the amplitudes of the small and large transients, respectively. The Ca_i alternans threshold was defined as the longest PCL required to produce a ΔCa_i peak > 0.1 [14]. The SDA threshold was defined as the longest PCL required to reach the alternans threshold on both sides of a nodal line. To estimate CV (at PCLs of 300, 200, 150, and 120 ms), we measured the distance and conduction time between the earliest activation point and two epicardial points in the normal and IR zones (Fig. 1), respectively. Wavelength (WL) was calculated by the formula $APD_{80} \times CV = WL$. The wet weight of the hearts was expressed as a percentage of the body weight of the rabbits.

2.6. Statistical methods

Values of continuous variables were expressed as mean ± standard deviation. Two-way repeated measure ANOVA comparisons were performed to evaluate the effects of piceatannol on APD_{80} (PCL at 300, 200 and 150 ms), CV (PCL at 300, 200, 150 and 120 ms) and WL (PCL at 300, 200 and 150 ms). The McNemar test was used to compare the VF inducibility at baseline and with piceatannol. A paired t-test was taken to indicate statistical significance in ERP, the mean maximum slope of APD restitution, Ca_i decay, the threshold of alternans and SDA before and after piceatannol treatment in the same hearts. An unpaired t-test was performed to compare the CV, APD_{80}, WL and Ca_i decay between the HF and control groups, and between the non-IR and IR zones. Differences were considered significant when $p < 0.05$.

3. Results

In the HF group, the mean LV ejection fraction was decreased from 61 ± 4% to 32 ± 3% after 4 weeks of rapid pacing ($p < 0.01$, $n = 11$). The wet weight of the hearts in the HF group (20 ± 3 g, $n = 11$) was significantly heavier than the control group (13 ± 2 g, $n = 11$, $p < 0.001$); and the ratio of heart weight/body weight was 0.66 ± 0.11% (HF group) vs. 0.40 ± 0.05% (control group, $p < 0.001$).

3.1. Electrophysiological responses to piceatannol administration

3.1.1. CV

Piceatannol decreased CV in both groups (Table 1). The HF group had slower CV than the control group at baseline (PCL of 300 ms ($p = 0.02$), 200 ms ($p = 0.08$), 150 ms ($p = 0.12$), and 120 ms ($p = 0.02$)) and with piceatannol (PCL of 300 ms ($p = 0.13$), 200 ms ($p = 0.06$), 150 ms ($p = 0.01$), and 120 ms ($p = 0.01$)). There was no significant difference of CV between the IR and the non-IR zones at baseline in both groups and with piceatannol in the control group (Table 2). However, the CV in the IR zone was significantly lower than the non-IR zone at PCLs of 150 ms ($p = 0.019$) and 120 ms ($p = 0.047$) in post-piceatannol failing hearts. As shown in Fig. 1A (example of isochronal map in the HF group), impulses propagated smoothly at longer PCLs (>200 ms), but it took longer to activate the reperfused region at shorter PCLs (<150 ms), especially after piceatannol infusion (bottom subpanels). At baseline, CV was decreased from 71 cm/s (PCL = 300 ms) to 54 cm/s (PCL = 120 cm) in the non-IR zone, and from 69 cm/s (PCL = 300 ms) to 54 cm/s (PCL = 120 cm) in the IR zone. After piceatannol infusion, CV was decreased from 61 cm/s (PCL = 300 ms) to 52 cm/s (PCL = 120 cm) in the non-IR zone, and from 61 cm/s (PCL = 300 ms) to 48 cm/s (PCL = 120 cm) in the IR zone in this heart. Fig. 1B shows an example in the control group. At baseline, CV was decreased from 80 cm/s (PCL = 300 ms) to 68 cm/s (PCL = 120 cm) in the non-IR zone, and from 79 cm/s (PCL = 300 ms) to 65 cm/s (PCL = 120 cm) in the IR zone. After piceatannol infusion, CV was decreased from 74 cm/s (PCL = 300 ms) to 60 cm/s (PCL = 120 cm) in the non-IR zone, and from 72 cm/s (PCL = 300 ms) to 54 cm/s (PCL = 120 cm) in the IR zone in this heart.