Original Article

Effects of Pinacidil on ST-T Wave Alternans During Acute Myocardial Ischemia in the *In-situ* Pig HeartIchiro WATANABE¹⁾ and Leonard S. GETTES²⁾¹⁾ Division of Cardiology, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan²⁾ Division of Cardiology, Department of Medicine, University of North Carolina at Chapel Hill, NC, USA

ST-T alternans during ischemia is associated with ventricular arrhythmias and reflects primary changes in repolarization, and changes secondary to 2:1 conduction block. We studied the effect of pinacidil, an ATP-sensitive K⁺ channel activator, on the incidence of ST-T alternans during ischemia. K⁺- and pH-sensitive electrodes and unipolar and bipolar electrodes were inserted into the midmyocardium in 9 open-chested pigs. Transmembrane action potentials were recorded from the epicardial surface. The carotid artery was shunted to the left anterior descending artery via a roller pump, and 25 μM pinacidil was infused. Prior to pinacidil administration, ST-T alternans occurred in all 9 pigs during control ischemia, at a mean onset time of 4 minutes 23 seconds ± 22 seconds. After pinacidil infusion, no ST-T alternans occurred during the 8-minute ischemic period. Furthermore, after pinacidil infusion, the final rise in extracellular K⁺ ([K⁺]_e) and the fall in extracellular pH (pH_e) were similar to the those at the onset of ST-T alternans under control conditions ([K⁺]_e: 7.6 ± 0.9 vs. 6.9 ± 0.9 mM; pH_e: 7.08 ± 0.78 vs. 7.05 ± 0.18). Activation delay was also similar with pinacidil administration compared with that at the onset of ST-T alternans, under the control condition (27 ± 18 ms vs. 34 ± 24 ms). With pinacidil, the action potential duration after 8 minutes of ischemia was decreased by 50% (from 250 ± 36 ms to 127 ± 15 ms). The complete suppression of ST-T alternans by pinacidil may reflect an increase in the diastolic interval.

Key words: acute ischemia, extracellular K⁺, extracellular pH, ST-T alternans, pinacidil

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Introduction

Unipolar direct-current electrograms recorded from acutely ischemic myocardium show increase in R wave amplitude, TQ segment depression, ST segment elevation, and T wave inversion¹⁾. These changes are related to disparities in the transmembrane action potential amplitude and duration between normal and acutely ischemic myocardium, and/or to activation delay in the ischemic zone. Beat-to-beat alternation of the unipolar waveform is also observed. This electrical alternans could be caused by change in the transmembrane action potential configuration^{2, 3)}, alternation in the degree of conduction delay, or both¹⁾. Another possibility, suggested by Hellerstein et al.⁴⁾, is that ST segment alternans occurs when certain parts of the ischemic myocardium fail to respond on alternate beats, i.e., 2:1 conduction block. Thus, it remains unclear whether electrical alternans in the setting of acute myocardial ischemia is related to changes in the transmembrane action potential configuration, to activation delay, or to partial 2:1 block. We have shown that ST-T alternans during acute myocardial ischemia consisted of 2 phases: an initial phase that occurs in the subendocardium during the early stage of ischemia and

may represent repolarization alternans and a second phase that occurs in the subepicardium during the later stage of ischemia and may represent depolarization alternans, and higher incidence of ST-T alternans frequently associated with ventricular fibrillation during ischemia⁵⁾.

We have reported that calcium antagonists decrease both the rise in extracellular K⁺ [K⁺]_e, and the fall in extracellular pH (pH_e), and previous studies have shown that calcium antagonists reduce the incidence of ST-T alternans during acute myocardial ischemia and decrease the incidence of ventricular fibrillation during ischemia^{6–10)}. We also have shown that ATP-sensitive K⁺ channel opener pinacidil decreases the rise in [K⁺]_e and the fall in pH_e that occur during acute myocardial ischemia¹¹⁾. However, the effect of ATP-sensitive K⁺ channel opener on the incidence of ST-T alternans during acute myocardial ischemia has not reported. Thus, we conducted the present animal model study to clarify the effect of ATP-sensitive K⁺ channel opener on the ST-T alternans that occurs during acute myocardial ischemia.

Methods

Experimental preparation: Animals used in this study were cared for according to the Position of the American

Heart Association on Research Animal Use and in accordance with accepted guidelines for the care and treatment of experimental animals at the University of North Carolina. Approval for the study was obtained from the University of North Carolina at Chapel Hill's Institutional Animal Use and Care Committee. The experimental preparation was similar to the preparation that we reported previously (Figure 1)^{12, 13}. Nine domestic pigs of either sex and weighing 30–50 kg were anesthetized with sodium pentobarbital (25 mg/kg), and this was followed by α -chloralose, as needed. Mechanical ventilation and supplemental oxygen were supplied via an endotracheal tube and a Harvard respirator. Arterial blood gases were monitored, and appropriate ventilator adjustments were made to maintain arterial $PO_2 > 80$ mmHg and a pH of 7.35–7.45. Catheters were placed in the femoral artery for blood pressure monitoring and blood sampling and in the femoral vein for blood sampling and administration of fluids and drugs. Core temperature was continuously monitored with a temperature probe (Yellow Springs Instrument Co., Yellow Springs, OH). Heating blankets were used to maintain the animals' body temperature at 36–37°C. The heart was exposed via median sternotomy and suspended in a pericardial cradle. A site midway along the length of the left anterior descending coronary

artery (LAD) and free of branches was selected for cannulation and dissected from surrounding tissue. The epicardial margin between ischemic and non-ischemic tissues was identified by brief occlusion of the vessel at this site, and 4–6 groups of ion-selective/unipolar and bipolar electrodes were placed at various locations in the center of the ischemic zone, defined as the region > 10 mm inside the visible cyanotic border, and in the normal (non-ischemic) zone.

After electrode placement, systematic heparin (10,000 U followed by 2,000 U/hour) was administered. A carotid artery-to-LAD shunt was created, as previously described, for later intracoronary administration of drugs^{12, 13}. Placement of the shunt in the LAD took approximately 2–3 minutes, but the LAD flow was stopped for 5 minutes because successive occlusions produced similar metabolic and electrical changes¹⁴. To ensure that all preparations were alike with respect to the duration of ischemia during shunt placement, ischemia was maintained for a total of 5 minutes during shunt placement in each pig. Perfusion of the distal LAD via the shunt was maintained at 1.2 mL/kg body weight/minute, which has been shown to provide a flow of 1.2–1.5 mL/g/heart tissue/minute¹⁵. Atrial pacing was used to increase heart rate. Arterial blood pressure and the lead II electrocardiogram were recorded continuously during each experiment with a 12-channel Graphtec Linearcorder (Graphtec, Yokohama, Kanagawa, Japan).

Fig. 1 Diagram of the experimental preparation used (see text for description).

LAD indicates left anterior descending coronary artery; LCX, left circumflex coronary artery.

Fashioning and insertion of ion-selective electrodes:

Ion-selective plunge electrodes were fashioned and calibrated as described previously¹⁶. Briefly, one end of Teflon-coated silver wire (0.007 in diameter) was chloridized by soaking it in sodium hypochlorite, covered with cellulose acetate-titanium dioxide sponge. K^+ - and H^+ ion sensitive electrodes were made by covering the sponge with a polyvinylchloride-valinomycin- and tridodecylamine-based membrane. Reference electrodes were fashioned in an analogous manner but lacked the ion-selective membrane. One K^+ -sensitive electrode along with 2 reference electrodes for recording bipolar electrograms in addition to $[K^+]_e$ measurement constituted 1 electrode group. One H^+ -sensitive electrode along with 1 reference electrode constituted 1 electrode group. Electrodes were calibrated before each experiment in standard solution (3 and 10 mmol/L KCl for K^+ -sensitive electrodes and pH 8 and pH 6 for H^+ -sensitive electrodes). Only electrodes that provided a stable baseline (drift less than 1 mV/hour) and 95–105% of the predicted Nernst slope (56–62-mV shift per decade change in K^+ activity at room temperature) were used. Four to six K^+ - and pH-sensitive electrodes were placed in the midmyocardium of the ischemic zone. After electrode insertion, the in-vivo performance of the K^+ - and H^+ -sensitive electrodes was tested by methods we have reported previously¹⁵. At the end of each ex-

periment, the electrodes were removed from the heart and re-tested in-vitro to confirm stable function throughout the experiment. The electrode group was threaded into a 19-gauge hypodermic needle, which was used to insert the group into the midmyocardium to a depth of 4–6 mm. The needle was then withdrawn, leaving the electrode group imbedded in the myocardium. As many as 6 electrode groups were used in each experiment.

Fashioning microelectrodes and signal recording:

Microelectrodes were used to record transmembrane potentials from subepicardial tissue layers. The microelectrodes were pulled from micropipettes with a standard microelectrode puller and filled with 3M KCl solution. The tips were long and flexible, and tip resistance was between 15 M Ω and 35 M Ω . The electrode was mounted on a tungsten wire (0.002 in diameter). The wire was connected to high-input resistance buffer amplifier. The microelectrode had a reference electrode consisting of silver/silver chloride wire placed as close as possible to it. Signals were differentially DC-amplified, continuously monitored on an oscilloscope, and recorded (Grathec Linearcorder) at 50 mm/second.

Use of transmural electrodes to determine transmural distribution of ST-T wave alternans: In 1 experiment, transmural distribution of ST-T alternans was determined by insertion of a 5-pole needle electrode at the center of the ischemic zone. The 5-pole needle electrode consisted of 5 electrodes placed at an interelectrode distance of 2 mm, allowing the recording of 5 unipolar DC electrograms (0–500 Hz) and 4 bipolar electrograms (50–500 Hz) from 2 adjacent electrode sites.

Experimental protocol: Fifty minutes after cannulation of the LAD, the first in a series of myocardial ischemias of 8 minutes' duration each was induced by abrupt cessation of flow through the LAD shunt during sinus rhythm and this ischemia was defined as control ischemia. We then infused 500 μ M/L pinacidil through the sidearm of the shunt at a rate calculated to produce a pinacidil concentration of 25 μ M/L in blood shunted to the LAD¹¹. The pinacidil infusion was initiated after 30 minutes of reperfusion and maintained for 20 minutes, at which time coronary flow was discontinued for 8 minutes (pinacidil ischemia).

Data collection and analysis: The amplified signals from all electrodes, along with a lead II electrocardiogram, were digitized with an analog-to-digital converter (Phoenix Data, Inc. Montgomery, PA, USA) and simultaneously sampled (1000 samples per second) every 15 seconds during myocardial ischemia by a MicroVAX II/GPX computer (Digital Equipment Corporation, Maynard,

MA, USA). $[K^+]_e$ values were calculated from measured millivolt changes according to the calibration curve of each electrode, and the systemic $[K^+]_e$ concentration determined from an arterial blood sample obtained immediately before the event. An activity coefficient of 0.746 was used in calculating the $[K^+]_e$ concentration. Signals from bipolar electrograms were filtered between 50 Hz and 500 Hz. Local activation was taken as the peak of the high frequency deflection on the local bipolar electrogram. Activation delay at each time point was calculated individually for each electrode by subtracting the baseline activation time (as referenced to an electrode in the non-ischemic zone) from that at each time point. Only sinus beats were analyzed. The number of acceptable electrodes in each experiment ranged from 3 to 5 for $[K^+]_e$ and from 2 to 3 for pH_e . Unipolar DC and bipolar electrograms were obtained only from the needles in which an acceptable K^+ -sensitive electrode was included.

Statistical analysis: Data are presented as mean $\pm$ SD unless otherwise indicated. Differences in values obtained between the control occlusion and pinacidil occlusion were assessed by Wilcoxon signed-rank test or Fisher's exact test, as appropriate. All statistical analyses were performed with StatView 5.0 software (SAS Institute, Cary, NC), and $P < 0.05$ was considered significant.

Results

A total of 18 coronary artery occlusions were studied in 9 pigs.

Heart rate did not differ significantly between control occlusion (113 ± 21 /minutes) and pinacidil occlusion (109 ± 33 /minutes) (Table 1). ST-T alternans occurred in all (9/9) pigs during control occlusion at mean time of 4 minutes 23 seconds $\pm$ 1 minute 06 seconds. After intracoronary administration of pinacidil, ST-T alternans did not occur in any pig (0/9) during the 8-minute occlusion (Table 1).

The $[K^+]_e$ concentration at the onset of ST-T alternans during control occlusion was 6.9 ± 0.3 mM, and at the end of the 8-minute pinacidil occlusion, the concentration was 7.6 ± 0.9 mM. These 2 concentrations did not differ significantly ($P = 0.118$) (Table 1). pH_e at the onset of the ST-T alternans during control occlusion was 7.05 ± 0.06 , and at the end of the 8-minute pinacidil occlusion, it was 7.08 ± 0.78 mM. This difference was not significant ($P = 0.912$) (Table 1).

With pinacidil administration, action potential duration at 90% repolarization (APD_{90}) decreased significantly (from 378 ± 24 ms before coronary occlusion to 310 ± 15 ms ($P < 0.001$), and APD_{90} shortening was further accelerated during coronary occlusion (from 250 ± 36 ms to 127 ± 15 ms at the end of the 8-minute ischemia) (Table 1).

Table 1 Values obtained during the 8-minute ischemia in the control and pinacidil conditions

	Control ischemia	Pinacidil ischemia	<i>P</i> value
Heart rate (bpm)	113 ± 21	109 ± 33	0.763
Mean blood pressure (mmHg)	100 ± 23	75 ± 20	< 0.001
ST-T alternans onset time	4'23" ± 1'06"	—	< 0.001
[K ⁺] _e (mM)	6.9 ± 0.9	7.6 ± 0.9	0.118
pH _e	7.05 ± 0.18	7.08 ± 0.78	0.912
APD ₉₀ (ms)	250 ± 36	127 ± 15	< 0.001
Activation delay (ms)	34 ± 24	27 ± 18	0.494

Values are shown as mean ± SD for the 9 animals. Note that ST-T alternans did not occur with pinacidil administration in any animal.

Representative changes in subepicardial transmembrane potential, unipolar DC electrograms, and subepicardial bipolar electrograms recorded from transmural electrodes during the 8-minutes of control ischemia are shown in Figure 2, left panel. Figure 2 middle panel shows changes in subepicardial transmembrane potential, unipolar DC electrograms, and subepicardial bipolar electrograms recorded from transmural electrodes during the 8-minute ischemia after administration of pinacidil. Figure 2 right panel shows changes in [K⁺]_e during the 8-minute ischemia before and after administration of pinacidil. ST-T alternans more obvious at endocardial sites appeared at 3 minutes 15 seconds of ischemia at a [K⁺]_e level of 9.5

mM, and ST-T alternans observed at 7 minutes 45 seconds of ischemia was more prominent at subepicardial sites accompanied by 2:1 subepicardial conduction block at a [K⁺]_e level of 13.1 mM. With administration of pinacidil, the APD₉₀ shortened from 298 ms to 210 ms before ischemia and then further to 120 ms at 8 minutes of ischemia. ST-T alternans was not observed during the 8 minutes of ischemia after administration of pinacidil, even though [K⁺]_e at 8 minutes of ischemia (9.4 mM) was similar to [K⁺]_e (9.5 mM) at the initiation of ST-T alternans during control ischemia. Representative unipolar DC electrograms obtained [K⁺]_e and pH_e levels were similar, i.e., at 3 minutes 45

Fig. 2 Effect of pinacidil on the rise in extracellular K⁺ ([K⁺]_e), transmural direct current (DC)-coupled unipolar electrograms, and subepicardial bipolar electrograms obtained in the control condition and with administration of pinacidil. ST-T alternans is seen during control ischemia, with early-phase ST-T alternans observed at subendocardial sites (black and white arrows in the middle recordings in the left panel and black arrows in the right recordings in the left panel) and later-phase ST-T alternans observed at subepicardial sites associated with 2:1 conduction block shown on the subepicardial bipolar electrogram. After pinacidil administration, ST-T alternans was not observed during ischemia even though [K⁺]_e was elevated to the same level seen when the initial ST-T alternans occurred in the control condition. APD₉₀ indicates action potential duration at 90% repolarization; subendo, subendocardium; subepi, subepicardium.

seconds (left panel) during control ischemia and at 8 minutes of ischemia after pinacidil administration, are shown in Figure 3. ST-T alternans was observed during control ischemia but was not observed during the 8 minutes of ischemia after pinacidil administration.

Discussion

In a previously reported study⁵⁾ that was based on the same experimental model used in the present study, we identified 2 types of ST-T alternans during acute myocardial ischemia: an initial ST-T alternans that occurred 4 minutes 15 seconds $\pm$ 12 seconds after the onset of ischemia, was most prominent in the subendocardium, and was independent of activation block, and a secondary ST-T alternans that was more obvious and greatest in the subepicardium. The secondary ST-T alternans began 4–8 minutes after the onset of ischemia and usually 30–60 seconds after the onset of the initial ST-T alternans, and it was consistently associated with a change in the shape of the bipolar electrogram or observance of 2:1 conduction block⁵⁾. We also observed the secondary ST-T alternans in 56% of animal hearts in which ventricular fibrillation occurred during ischemia⁵⁾. Beat-to-beat change in the unipolar waveform has been associated with the development of early post-occlusion ventricular arrhythmias^{17–19)}. There have been a few animal studies conducted to examine the effect of anti-ischemic drugs on the occurrence of ST-T alternans during acute myocardial ischemia. Hashimoto, et al showed that calcium antagonists de-

creased the incidence of ST-T alternans during acute myocardial ischemia, and Hayakawa, et al showed that diltiazem decreased the incidence of ST-T alternans and ventricular tachycardia/fibrillation during acute myocardial ischemia^{8–10, 20)}. We have shown, by measuring extracellular K^+ , pH, and intramyocardial conduction time in an animal model, the anti-ischemic effect of verapamil^{5–7)}. We have also shown the anti-ischemic effect of ATP-sensitive K^+ channel opener pinacidil in the same experimental model¹¹⁾. However, whether this ATP-sensitive K^+ channel opener has an effect on the incidence of ST-T alternans during acute myocardial ischemia has not been reported. In the present study, we found that pinacidil completely suppressed ST-T alternans during the 8 minutes of ischemia.

The suppression of ST-T alternans by pinacidil can be explained by its anti-ischemic effect, which is evidenced by suppression of both the rise in extracellular K^+ and the fall in extracellular pH¹¹⁾. Furthermore, ST-T alternans was not observed after administration of pinacidil when the $[K^+]_e$ and pH_e levels were similar to those at the onset of ST-T alternans during control ischemia. Therefore, marked APD shortening during ischemia induced by pinacidil may also contribute to the suppression of ST-T alternans effected by pinacidil. Direct APD shortening effect of pinacidil in the absence of ischemia may also contribute to the suppression of ST-T alternans during ischemia. Verapamil has also been shown to lessen the activation delay, decrease the action potential amplitude,

Fig. 3 Unipolar electrograms recorded at similar extracellular K^+ ($[K^+]_e$) and extracellular pH (pH_e) levels.

Note that in this experiment, the ST-T alternans observed during control ischemia (indicated by the black and white triangles) was not observed after pinacidil administration despite the similarity in $[K^+]_e$ and pH_e levels between the control and pinacidil ischemias.

DC indicates direct current.

and shorten the APD leading to ST segment elevation in the ischemic myocardium at $[K^+]_e$ levels that are similar between control and verapamil ischemia^{21, 22)}. Unlike verapamil, pinacidil has been shown to produce both antiarrhythmic^{23–25)} and proarrhythmic activity^{26–28)} during myocardial ischemia. In the present study, no lethal ventricular tachyarrhythmia was observed during either control or pinacidil ischemia.

Study Limitations: The present study was limited by the small number of experiments, by the fact that only 1 dose of pinacidil was used, and by the fact that ventricular tachycardia/fibrillation did not occur during control or pinacidil ischemia. A future study consisting of a greater number of experiments, various doses of pinacidil, and longer coronary artery occlusion times is needed to evaluate the effects of pinacidil on arrhythmogenesis during acute myocardial ischemia. Second limitation was that we only tested pinacidil, therefore it was unclear whether the results were applicable to other ATP-sensitive K^+ channel openers.

Conclusion: Our experiments indicate that ATP-sensitive K^+ channel opener pinacidil suppresses the ST-T alternans that occurs during acute myocardial ischemia. The anti-ischemic effect of pinacidil and marked pinacidil-induced shortening of the APD may explain the mechanism of suppression.

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Disclosures

The authors have no conflicts of interest to disclose.

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