Effects of Propranolol and Verapamil on Changes in TQ and ST Segment Potentials During Graded Coronary Flow Reduction in a Porcine Myocardial Ischemia Model

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SUMMARY

Acute myocardial ischemia causes TQ depression and ST elevation. However, the effects of cardioprotective drugs such as β-blockers and Ca++-antagonists on the extent of TQ depression, ST elevation, and myocardial ischemic injury are not fully understood.

We created a carotid-coronary shunt in 30 pigs, and extracellular K+ ([K+]e), TQ, and ST segments were recorded simultaneously with K+-selective plunge electrodes placed in the left anterior descending artery (LAD) distribution during graded LAD flow reduction before and after administration of propranolol or verapamil. Unipolar DC-coupled electrograms were recorded from the reference pole of the K+-selective plunge electrodes. The microvolt readings from the K+-selective electrodes were converted to [K+]e, and then to the changes in potassium equilibrium potential (ΔEK). The shunted LAD flow was reduced in a stepwise fashion at 5-minute intervals.

TQ segment depression at the similar ΔEK was not affected by propranolol or verapamil. However, ST segment elevation was reduced by propranolol but exacerbated by verapamil at the similar ΔEK.

TQ-ST changes recorded by AC coupled ECG are not a reliable index of ischemia and therefore cannot be used to evaluate the effects of drugs that might affect the electrophysiologic properties of ischemic myocardium. (Int Heart J 2017; 58: 428-434)

Key words: Unipolar DC electrogram, Extracellular K+, β blocker, Calcium antagonist

Fatal arrhythmias are not uncommon in the early stage of myocardial infarction. Harris, et al first reported extracellular potassium ([K+]e) accumulation as a major determinant of these arrhythmias on the basis of concentrations measured in blood samples drawn from coronary veins draining the ischemic region. Direct measurement was later made possible with the introduction of potassium-selective electrodes. Hill, et al reported the change in extracellular myocardial potassium ([K+]e), recorded by potassium-sensitive electrodes to be an excellent marker of ischemia in a no-flow model. Cellular K+ loss is mainly due to an increase of K+ efflux via the time-independent K current and ATP-dependent K+ current rather than to a decrease of K+ influx. We have reported that [K+]e, extracellular pH (pHe), and TQ-ST segment changes provide the most sensitive means of detecting myocardial ischemia and that verapamil, but not propranolol, shifts the threshold flow for the rise in [K+]e, and fall in pHe downward.

TQ depression and ST elevation on DC-coupled extracellular electrocardiograms (ECGs) obtained during acute myocardial ischemia have been shown to be the result of diastolic and systolic currents of injury between the normal side of the border zone and ischemic tissue. Previous experimental and clinical studies used sigma ST segment elevation, ie, total ST segment elevation in all leads, as an index of myocardial ischemic injury. However, the relation between TQ segment depression and [K+]e elevation during acute myocardial ischemia has been shown to change dynamically, mostly likely because of factors besides [K+], altering resting membrane potential, tissue anisotropy, and changes in extra- and intracellular resistances during ischemia. In addition, the extent of ST segment elevation is influenced by the local activation delay and action potential amplitude of the ischemic myocardium. Potassium equilibrium potential is known to be virtually identical to the resting membrane potential of the acutely ischemic myocardium. We, therefore, studied the effects of propranolol and verapamil on TQ and ST segment changes during graded coronary flow reduction in situ pig hearts, and we compared the TQ and ST segment changes that occurred in relation to the change in the potassium equilibrium potential (ΔE_K) during ischemia before and after administration of either of these drugs.
METHODS

In-situ pig heart preparations: Preparation of hearts in open-chest pigs was similar to that reported previously. Twenty-seven domestic swine 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 oxygen supplementation were provided via an endotracheal tube and a Harvard respirator. Arterial blood gases were monitored, and appropriate ventilator adjustments were made to maintain an arterial PO2 of > 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, USA). A heating blanket was used to maintain each animal’s 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. After brief occlusion of the left anterior descending artery (LAD) at this site, the epicardial margin between ischemic and non-ischemic tissues was identified, and 4 - 6 pairs 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, systemic heparin (10,000-U bolus followed by 2,000 U/hour) was administered. A carotid artery-to-LAD shunt was created, as previously described. Placement of the shunt took approximately 2 - 3 minutes, but the LAD flow was stopped for 5 minutes because successive occlusions produced similar metabolic and electrical changes.

This carotid-coronary shunt was routed through a constant-flow roller pump, which permitted controlled reduction in flow from a control value of 1.2 - 1.5 mL per kg body weight/minute (30 - 50 mL/minute) to zero. Heparin (3,000-U bolus followed by 7,000 U/hour) was administered to ensure cannula patency. Atrial pacing was used to maintain heart rate at 100 beats/minute. Arterial blood pressure and the lead II ECG were monitored in vivo for at least 60 minutes after placement of the K+-sensitive electrodes. Coronary blood flow through the roller pump was then reduced in a stepwise fashion at 5-minute intervals as follows: 50, 40, 30, 20, 15, 10, 5, 2.5, and 0 mL/minute, followed by a return to the control flow rate. In the verapamil group, an intravenous loading dose of 0.2 mg/kg verapamil was initiated 20 minutes after first graded flow reduction over a 20-minutes period. This was followed by a constant infusion of 6.5 μg/kg/minute. In the propranolol group, an intravenous loading dose of 0.4 mg/kg propranolol was initiated 40-minutes after first graded flow reduction and administered over a 5 minute period. This was followed by a constant infusion of 3 μg/kg/minute. In all groups, the second flow reduction was performed 50 minutes after first flow reduction.50 [K+] and TQ and ST segment data were collected at the end of each coronary flow reduction, ie, 5 minutes after each coronary flow reduction.

Experimental protocol: The preparation was allowed to stabilize for at least 60 minutes after placement of the K+-sensitive electrodes. Coronary blood flow through the roller pump was then reduced in a stepwise fashion at 5-minute intervals as follows: 50, 40, 30, 20, 15, 10, 5, 2.5, and 0 mL/minute, followed by a return to the control flow rate. In the verapamil group, an intravenous loading dose of 0.2 mg/kg verapamil was initiated 20 minutes after first graded flow reduction over a 20-minutes period. This was followed by a constant infusion of 6.5 μg/kg/minute. In the propranolol group, an intravenous loading dose of 0.4 mg/kg propranolol was initiated 40-minutes after first graded flow reduction and administered over a 5 minute period. This was followed by a constant infusion of 3 μg/kg/minute. In all groups, the second flow reduction was performed 50 minutes after first flow reduction.50 [K+] and TQ and ST segment data were collected at the end of each coronary flow reduction, ie, 5 minutes after each coronary flow reduction.

Data collection and analysis: The amplified signals from all electrodes and the lead II ECG were digitized with an analog-to-digital converter (Phoenix Data Inc., Phoenix, AZ, USA) and simultaneously sampled (1000 samples per second) every
15 seconds during myocardial ischemia with a MicroVAX II/GPX computer (Digital Equipment Corp., Maynard, MA, USA). Simultaneous acquisition of the changes in TQ and ST segment potentials measured 100 msec after the peak of the R wave of the unipolar electrograms, ie, changes in TQ and ST segment potentials from those measured at the control flow rate (ΔTQ and ΔST) was accomplished by using the K\textsuperscript+ reference electrode as one pole for recording of a unipolar, DC-coupled electrogram referenced to an Ag/AgCl-electrode attached to the aortic root (Figure 2). The microvolt readings from the K\textsuperscript+ -sensitive electrodes were first converted to [K\textsuperscript+]e and then to ΔE\textsubscript{k}\textsuperscript{10}. Changes in TQ potential and ST potential were compared at flow rates for which ΔE\textsubscript{k} values were similar and then after administration of propranolol or verapamil.

**Statistical analysis:** Data are presented as the mean ± SD unless otherwise indicated. Differences in values were analyzed by the Wilcoxon signed-rank test. All statistical analyses were performed with StatView 5.0 software (SAS Institute, Cary, NC), and P < 0.05 was considered significant.

**RESULTS**

ΔE\textsubscript{k} during the controlled flow reduction was 15.08 ± 5.87 mV at a mean coronary flow of 4.8 ± 2.6 mL/minute and then with verapamil administration was 15.48 ± 6.03 mV (P = 0.184) at mean coronary flow of 2.3 ± 1.5 mL/minute (P = 0.003). ΔTQ during the controlled flow reduction was –4.57 ± 1.91 mV and then with verapamil administration was –4.47 ± 1.78 mV (P = 0.883). ΔST during the controlled flow reduction was +3.57 ± 2.38 mV and then with verapamil administration was +6.67 ± 3.33 mV (P = 0.007 (Figure 3)). ΔE\textsubscript{k} during the controlled flow reduction was 18.48 ± 5.25 mV at a mean coronary flow of 5.4 ± 3.5 mL/minute and then with propranolol

**Figure 2.** Diagrams of K\textsuperscript+ -sensitive electrode construction (left) and diagram of simultaneous [K\textsuperscript+]e and TQ potential measurements (right).

**Figure 3.** Bar graphs showing the change in potassium equilibrium potential (ΔE\textsubscript{k}), change in TQ segment potential (ΔTQ), and change in ST segment potential (ΔST) under the control (controlled graded coronary flow reduction) condition and then under verapamil administration and recorded from the mid-myocardium of the ischemic myocardium when ΔE\textsubscript{k} values were similar.
administration was $18.43 \pm 5.36$ mV ($P = 0.981$) at a mean coronary flow of $5.6 \pm 3.4$ mL/minute ($P = 0.888$). $\Delta TQ$ during the controlled flow reduction was $-5.38 \pm 1.42$ mV and then with propranolol administration was $-5.71 \pm 1.60$ mV ($P = 0.598$). $\Delta ST$ during the controlled flow reduction was $+6.42 \pm 2.48$ mV and then with propranolol administration was $+5.46 \pm 2.28$ mV ($P = 0.010$) (Figure 4).

A representative experiment of the verapamil group is shown in Figure 5. In this experiment, intramyocardial and epicardial DC-coupled unipolar electrograms were recorded. We found on the intramyocardial and epicardial DC-coupled electrograms obtained in a representative experiment that $\Delta E_k$ values were similar between the controlled flow reduction (15.7 mV at a coronary flow rate of 5 mL/minute) and the flow reduction with verapamil administration (17.3 mV at a coronary flow rate of 2.5 mL/minute). Mid-myocardial $TQ$ depression was $-5.0$ mV and $-5.0$ mV during the controlled flow reduction and with verapamil administration, respectively, and ST elevation was $+4.5$ mV and $+8.5$ mV, respectively (Figure 5, upper tracing). Epicardial $TQ$-$ST$ segment elevation was $+7.5$ mV and $+22.0$ mV during the controlled flow reduction and with verapamil administration, respectively (Figure 5, lower tracing). We also found on the intramyocardial and epicardial DC-coupled electrograms obtained in a representative experiment that $\Delta E_k$ values were similar between the controlled flow reduction (22.7 mV at coronary blood flow of 2.5 mL/minute) and the flow reduction with propranolol administration (25.4 mV at coronary flow of 2.5 mL/minute). Mid-myocardial $TQ$ depression was $-8.0$ and $-6.5$ mV during the controlled flow reduction and with propranolol administration, respectively,
and ST elevation was +10.5 mV and +11.5 mV, respectively (Figure 6, upper tracing). Epicardial TQ-ST segment elevation was +11.5 and +6.0 mV during the controlled flow reduction and with propranolol administration, respectively (Figure 6, lower tracing). In an experiment, we observed a decrease in ST segment elevation during no-flow ischemia compared to that in very low-flow ischemia, despite smaller increases in ΔE_k and ΔTQ (Figure 7).

**Discussion**

In this study, we compared the magnitude of changes in TQ and ST segment potentials in the mid-myocardium at the center of the ischemic zone during graded LAD flow reduction before and after administration of verapamil and before and after administration of propranolol in *in situ* pig hearts. Neither verapamil nor propranolol affected change in the TQ segment when ΔE_k values were similar. Verapamil, however, exaggerated change in the ST segment, and propranolol attenuated...
changes in the ST segment when ΔEk values were similar. Previous experimental and clinical studies used sigma ST segment elevation as an index of myocardial ischemic injury. We reported previously that verapamil decreases the rise in [K+]i, and fall in pH, during both no-flow ischemia and low-flow ischemia, that propranolol does not affect the rise in [K+]i, or fall in pH during low-flow ischemia when the heart rate is held constant, and that propranolol does not affect the maximum rise in [K+]i during no-flow ischemia. Furthermore, verapamil lessens activation delay during ischemia, whereas propranolol aggravates the delay. The effects of verapamil on local activation have been shown to persist when either [K+]i, or pH values are matched, and this may be explained in part by a lessening of ionic inhomogeneity during ischemia. Verapamil has been shown to enhance ischemia-induced action potential shortening in vitro. In a study conducted by Botti, et al verapamil decreased sigma ST segment elevation in patients with acute myocardial infarction, despite a transient early increase in some patients. El-Khoury, et al demonstrated a decrease in surface ECG ST segment elevation during balloon occlusion after intracoronary administration of verapamil. In a study conducted by Kupersmith, et al propranolol aggravated activation delay in the ischemic zone and prolonged action potential duration in the ischemic zone at a fixed heart rate. Bodenheimer, et al reported that propranolol yielded a significant decrease in sigma ST, consistent with a decrease in heart rate, but when heart rate was increased by atrial pacing to the control occlusion rate, sigma ST returned to the control occlusion level. Hayase, et al demonstrated that the /β blocker tilisolol briefly reduced the ST segment elevation and TQ segment depression induced by ischemia in an in vivo canine experiment without atrial pacing.

Our 2 experiments shown in Figure 5 and Figure 6 revealed greater epicardial unipolar electrogram changes than mid-myocardial changes under verapamil and propranolol administration. It has been reported that ST segment elevation, as recorded with condenser-coupled AC amplifiers, can be due to both diastolic (TQ) depression and true ST segment elevation. The diastolic baseline depression in the extracellular electrogram is caused by loss of resting membrane potential in ischemic cells, leading to a diastolic current of injury between healthy cells and ischemic cells, which renders the extracellular space in the ischemic area negative with respect to the normal area. The mechanism responsible for the ST elevation is a shortening of the ischemic action potential. In the absence of activation delay of the ischemic cells, a systolic current of injury would be expected to flow between the ischemic and normal areas, making the ischemic extracellular space positive with respect to the normal extracellular space. However, action potential amplitude and activation delay also affect ST segment elevation. Verapamil has been shown to lessen the activation delay, decrease the action potential amplitude, and shorten the action potential duration in the ischemic myocardium, which may result in a greater systolic current of injury without further changes in Ek and the TQ segment during ischemia. Propranolol has been shown to aggravate activation delay in the ischemic zone during both low-flow and no-flow ischemias and to prolong action potential duration in the ischemic zone which may result in a systolic current of injury without further changes in Ek and the TQ segment during ischemia. Therefore, the cardioprotective effect of verapamil during acute myocardial ischemia judged from ST segment might reflect less accumulation of [K+], leading to less TQ-segment depression and the cardioprotective effect of propranolol during acute myocardial ischemia may be due mainly to its negative chronotropic effects. Lethal ventricular tachyarrhythmias during acute myocardial ischemia have been related to ST-T alternans due to depolarization and repolarization alternans and spatial heterogeneity of the APD restitution, and extracellular K+ gradient across the ischemic border zone leading to systolic and diastolic injury current, and verapamil lessened the standard deviation of Ek of the center and margin of ischemic myocardium during early stage of the ischemia. We demonstrated that the ATP-sensitive K+ channel opener pinacidil lessened the rise in extracellular K+ during acute myocardial ischemia mainly by a marked decrease of the action potential duration of the ischemic myocardium. Recently, intracoronary administration of verapamil and nicorandil, an ATP-sensitive K+ channel opener, has been shown to be effective for the treatment of slow/no flow phenomenon in the acute coronary syndrome. Therefore, intracoronary administration of verapamil and ATP-sensitive K+ channel opener before balloon occlusion of the coronary artery or in the presence of acute myocardial ischemia may aggravate ST segment elevation of the AC-coupled ECG in spite of the anti-ischemic effects of these drugs.

Study limitation: We did not simultaneously measure activation time from bipolar AC-coupled electrograms at sites adjacent to the unipolar DC electrogram recording sites, and we did not record action potentials from the ischemic myocardium. Therefore, the effects of verapamil and propranolol on the activation time and action potential duration of the ischemic myocardium were not shown in this study.

Conclusions: TQ-ST changes recorded on the AC-coupled ECGs cannot be used to evaluate the extent of severity of the ischemia by drugs that might affect the electrophysiologic properties of ischemic myocardium.

Disclosure

The authors have no conflicts of interest to declare.

References


Watanabe I, Gettes LS. Initial and secondary ST-T alternans during acute myocardial ischemia in the in-situ pig heart. Int Heart J 2016; 57: 327-35.


