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Improved Left Ventricular Function and Reduced Necrosis after Myocardial Ischemia/Reperfusion in Rabbits Treated with Ranolazine, an Inhibitor of the Late Sodium Channel

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Nonstandard abbreviations: CAO - coronary artery occlusion; RMBF - regional myocardial blood flow

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Abstract

Ranolazine is an inhibitor of the late sodium channel, and via this mechanism decreases sodium-dependent intracellular calcium overload during ischemia and reperfusion. Ranolazine reduces angina, but there is little information on its effects in acute myocardial infarction. The aim of this study was to test the effects of ranolazine on left ventricular (LV) function and myocardial infarct size after ischemia/reperfusion in rabbits**.** Ten minutes before coronary artery occlusion (CAO), anesthetized rabbits were assigned to vehicle (n=15) or ranolazine (2 mg/kg I.V. bolus plus 60 μg/kg/min I.V. infusion, n=15). Hearts received 60 min of CAO and 3 hours reperfusion. CAO caused LV dysfunction associated with necrosis. However at the end of reperfusion, rabbits treated with ranolazine had better global LV ejection fraction (0.42 ± 0.02 vs. 0.33 ± 0.02, $p<0.007$) and stroke volume (1.05 ± 0.08 ml vs. 0.78 ± 0.07 ml, $p<0.01$) compared with vehicle. The fraction of the LV wall that was akinetic or dyskinetic was significantly less in the ranolazine group at 0.23 ± 0.033 vs. 0.34 ± 0.03 in vehicle treated, $p<0.02$. The ischemic risk region was similar in both groups; however infarct size was significantly smaller in the treated group (44 ± 5 vs. 57 ± 4% vehicle, $p<0.04$). There were no significant differences between groups in heart rate, arterial pressure, LVEDP or maximum positive or negative dP/dt. In conclusion, the results of this study show that ranolazine provides protection during acute myocardial infarction in this rabbit model of ischemia/reperfusion. Ranolazine treatment led to better ejection fraction, stroke volume and less wall motion abnormality after reperfusion, and less myocardial necrosis.

Introduction

Ranolazine is known to be an effective antianginal agent in humans (Jain et al., 1990; Thadani et al., 1994; Rosseau et al., 2005; Pepine et al., 1999; Chaitman et al., 2004a; Chaitman et al., 2004b). Although an early theory held that ranolazine functioned by partially inhibiting fatty acid oxidation, new research provides evidence that the cardioprotective actions of ranolazine (at least at the doses currently being used) are not related to inhibition of fatty acid oxidation but are instead related to its effect of inhibiting the late sodium channel (I_{Na}) in cardiac cells (Song et al., 2004; Antzelevitch et al., 2004; Belardinelli et al., 2004). Through this effect as a selective inhibitor of the late I_{Na}, ranolazine reduces the rise in intracellular sodium, and ultimately the influx of calcium, that can accompany myocardial ischemia (Belardinelli et al., 2006).

Ranolazine inhibits late I_{Na} of ventricular myocytes with a potency (IC50 value) that varies from 5 to 21 μM depending on the experimental preparation, conditions and species (Antzelevitch et al.,2004; Song et al., 2004; Fredj et al., 2006; Undrovinas et al.,2006). The IC50 of ranolazine to inhibit peak I_{Na} of canine ventricular myocytes is 294 μM; hence ranolazine is a significantly more potent inhibitor of late than peak I_{Na}. In addition, ranolazine inhibits the rapid inward rectifying potassium current, I_{Kr} with a potency of 11.2 μM, and is a weak inhibitor of the L-type calcium inward current ($I_{Ca,L}$) and of the Na+/Ca++ exchanger current with potencies of 296 μM and 91 μM, respectively (Antzelevitch et al.,2004). Thus, within or slightly above ranolazine's therapeutic plasma concentration only late I_{Na} and I_{Kr} are likely to be significantly inhibited.

The components of the sodium channel can be amplified by ischemic metabolites (Undrovinas et al., 1992; Wu and Corr, 1994) and by oxygen free radicals released at reperfusion (Ward and Giles, 1997). Although the amplitude of the sodium influx via the late sodium channel represents less than 1% of the peak sodium influx, a substantial increase of sodium into the cell can occur during this phase. An increase in intracellular sodium concentration via the late

channel can lead to subsequent intracellular calcium overload via the Na+/Ca2+ exchanger. Calcium overload in myocytes then causes mechanical dysfunction and cell death. The amount of sodium overload is a determinant of cardiac function after reperfusion (Imahashi et al., 1999).

As an inhibitor of the late sodium channel, ranolazine might prevent or reduce excess sodium accumulation in cells and the subsequent, sodium-dependent calcium overload at reperfusion. Ranolazine has been shown in several clinical trials to reduce the pain frequency of angina and to prolong exercise time in patients with coronary artery disease (Jain et al., 1990; Thadani et al., 1994; Rosseau et al., 2005;Pepine et al., 1999; Chaitman et al., 2004a; Chaitman et al., 2004b). It has been shown to be safe and effective used alone (Chaitman et al., 2004a), or in combination with other agents used in the treatment of angina (Chaitman et al., 2004b). Ranolazine has recently been approved by the FDA for treatment of chronic angina in patients who fail to respond to other angina drugs. In contrast with other anti-anginal treatments that work by decreasing indices of cardiac work, ranolazine does not affect heart rate or blood pressure.

Ranolazine has been shown to reduce some indices of ischemic damage in animal models. For example, ranolazine reduced myocardial CK release in isolated guinea pig hearts (Clarke et al., 1993) and baboons (Alley and Alps, 1990), but in a study in dogs, no reduction in infarct size was found (Black et al., 1994). Ranolazine also improved left ventricular developed pressure after global ischemia in an isolated perfused rabbit heart (Gralinski et al., 1994). However data on the effects of ranolazine on cardiac function and anatomic infarct size in an intact animal model of regional ischemia induced by coronary artery occlusion are limited. Therefore the goal of this study was to assess whether ranolazine reduces anatomic myocardial infarct size and improves regional and global left ventricular (LV) function in the setting of acute myocardial infarction.

Methods

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The rabbits used in this study were maintained in accordance with the policies and guidelines of the Position of the American Heart Association on research animal use (American Heart Association, 1985) and the National Research Council: Guide for Care and Use of Laboratory Animals (1996). The Association for Assessment and Accreditation of Laboratory Animal Care International accredits Good Samaritan Hospital. The protocol was approved by the Institutional Animal Care and Use Committee of Good Samaritan Hospital.

Surgical preparation

Male New Zealand White rabbits (2.1-3.5 kg) were anesthetized with an intramuscular injection of a mixture of ketamine (approximately 75 mg/kg) and xylazine (5 mg/kg). Pentobarbital anesthesia was given intravenously during the study as required to maintain a deep level of anesthesia. The rabbits were intubated and mechanically ventilated with oxygen-enriched air. Fluid-filled catheters were inserted into the left jugular vein to administer fluids and drug treatment, into the left carotid artery to measure systemic pressure and to take a reference blood sample during regional myocardial blood flow measurement, and into the left ventricle via the right carotid artery to measure pressures and to inject contrast medium during ventriculography. The chest was opened through the left fourth intercostal space. The pericardium was incised and the heart exposed. Near the base of the heart the first large antero-lateral branch of the circumflex artery, or the circumflex artery itself, was encircled with a 4-O silk suture. Coronary occlusion in this region normally results in ischemia of a large territory of the antero-lateral and apical ventricular wall. The ends of the suture were threaded through a piece of tubing, forming a snare that was tightened to occlude the artery. A temperature probe was inserted into the rectum, and body temperature was maintained with a heating pad.

Dose-finding study

In a pilot study conducted before the present study, ranolazine blood levels were measured in 5 rabbits after a bolus dose of ranolazine, 2 mg/kg injected over 60 seconds, and an

infusion at a rate of 60 μg/kg/min, as used in the present study. Plasma samples were taken between 5 and 240 minutes from giving the bolus dose.

Experimental Protocol

After surgical preparation and a 15-minute stabilization period, baseline hemodynamic parameters and temperature were obtained. The rabbits were randomized to receive ranolazine (2 mg/kg bolus, injected over 60 seconds, plus 60 μg/kg/min, n=15) or an equivalent amount of vehicle, n=15. (The investigator was blinded to treatment until the completion of the entire study.) Treatment was initiated 10 minutes before coronary artery occlusion (CAO) and continued throughout reperfusion. Ten minutes after the start of treatment, hemodynamic variables were recorded and, the coronary artery was occluded by tightening the snare. The rabbits were then subjected to 60 minutes of coronary artery occlusion followed by three hours of reperfusion. Hemodynamic parameters were monitored and recorded at baseline, before CAO, at 15, 29 and 59 minutes of occlusion, and at 30, 60, 90, 120 and 165 minutes of reperfusion. Body temperature was maintained using a heating pad.

At the end of the reperfusion period, ventriculography was performed and regional myocardial blood flow was measured. The coronary artery was re-occluded and the ischemic risk region was delineated with 4 ml of a 50% solution of Unisperse blue dye (Ciba-Geigy, Hawthorne, NY) injected into the left atrium. The deeply anesthetized rabbit was killed by an injection of KCl (12 mEq) into the left atrium, and the heart was excised.

Hemodynamic measurements and rectal temperature

Heart rate, LV systolic pressure, LV end-diastolic pressure (LVEDP) and maximum positive and negative first derivative of LV pressure (dP/dt_{max} and dP/dt_{min}) were measured using fluid-filled catheters inserted into the carotid artery and into the left ventricle. Data were digitized and recorded at a sampling rate of 1K/sec using an ADI (Advanced Digital Instruments, Grand Junction, CO) system. Three consecutive cycles were averaged.

Assessment of LV dysfunction

A left ventriculogram was performed at the end of the reperfusion period in the lateral position using a XiScan fluoroscopic system. Three ml of contrast medium were injected into the left ventricle, and the image of the left ventricular cavity was recorded on video tape. Later measurements of end-systolic and end-diastolic volumes, ejection fraction and stroke volume were measured in three consecutive beats and the results averaged. Wall motion abnormality was also assessed from the ventriculogram. End-diastolic and end-systolic images of the LV cavity were traced and superimposed. Distances along the anterior circumference that were akinetic (overlapping diastolic and systolic images) or dyskinetic (systolic image bulging beyond the diastolic image) were measured and expressed as a fraction of the diastolic circumference.

Regional myocardial blood flow (RMBF)

RMBF was measured using approximately 2 x 10^6 radioactive microspheres (PerkinElmer Life Sciences, Boston, MA), 15μ, labeled with ^{141}Ce or ^{103}Ru. Microspheres were injected into the left atrium through a left atrial catheter, inserted at the end of the study, and a reference blood sample was obtained from the carotid artery. Tissue samples were cut from the risk region (determined by the absence of the blue dye) and from non-ischemic regions. The samples were weighed and counted together with the reference blood samples in a computerized gamma well counter (Canberra, System S100, Meriden, CT). RMBF was computed and the results were expressed as ml/min/g. The relative return of blood flow to the previously ischemic region at the end of the reperfusion period was computed as: RMBF in the risk zone/ RMBF in the non-ischemic zone.

Analysis of risk zone and necrosis

The heart was sliced transversely into 6-8 sections and photographed. The slices were photographed to identify the area at risk (no blue dye). The slices were then incubated in a 1% solution of triphenyltetrazolium chloride (Sigma-Aldrich Co., St. Louis, MO) for 15 minutes, immersed in formalin, and re-photographed. The photographs were enlarged and traced. The areas of ischemic risk zone (no blue dye) and normally perfused regions (stained blue), and the areas of necrotic (yellowish white) and non-necrotic regions (stained bright red) in each slice were

quantitated by digitized planimetry. The areas in each slice were multiplied by the weight of that slice, and the results were summed to obtain the weights of the risk and infarcted areas. Ischemic risk zone was expressed as: the weight of the risk zone / the weight of the left ventricle. Infarct size was expressed as the percent of the risk zone that was necrotic.

Statistical Analyses

Data were tabulated and calculated using Excel work sheets. All statistical analyses were performed using SAS (Version 6.04, Cary, NC). Changes in hemodynamic variables over time and between groups were analyzed by repeated measures analysis of variance. Left ventricular weight, infarct size, area at risk, and RMBF were compared using Student's t test, as were measurements obtained from the LV angiogram. Analysis of covariance (ANCOVA) was used to test for a group effect on the regression models of 1) ejection fraction versus extent of necrosis and 2) relative blood in the risk region versus the extent of necrosis. Data are expressed as mean ± SEM.

Results

Ranolazine plasma levels

Before the present study, the dosing regimen and blood levels of ranolazine were studied in 5 rabbits. At 5 minutes after administration of the bolus dose (2 mg/kg) and starting the infusion (60 µg/kg/min), blood levels of ranolazine had reached 3 to 5 µM (average 4.5 ± 0.5µM). Over the time period of the study (240 minutes), ranolazine concentrations in the 5 animals ranged between an average of 4.5 ± 0.5 and 8.8 ± 0.5 µM. These blood levels are comparable to the therapeutic range in humans (Chaitman et al., 2004a).

Hemodynamics

No significant differences between the two groups in basal heart rate, mean arterial pressure, LVEDP or peak positive or negative dP/dt were observed. (Fig 1). No substantial changes in heart rate were noted throughout the study period. Mean arterial pressure decreased during coronary artery occlusion and reperfusion in both groups with no significant differences

between groups. LVEDP increased during coronary artery occlusion and recovered during reperfusion to a similar extent in both groups. There was a time related effect in changes in both peak positive and peak negative dP/dt (absolute values decreased) during ischemia and reperfusion that was similar in both groups.

Risk Zone and Infarct Size

There were no significant differences in body weight, LV weight (data not shown) or extent of ischemic risk zone in the two groups. Risk zone, expressed as a percent of LV weight was 35 ± 3% in the vehicle group and 30 ± 2% in the ranolazine group (not significant). However, infarct size, expressed as a percent of the risk zone, was 57± 4% in the vehicle group and 44± 5% in ranolazine treated hearts ($p = 0.04$). Thus ranolazine administration reduced infarct size compared with vehicle.

Effect of ranolazine on LV dysfunction after reperfusion

At the end of the reperfusion period, a left ventriculogram was performed to compare LV cavity volumes during end-diastole and end-systole, ejection fractions and stroke volumes in the two groups. Mean ejection fraction was significantly better in the group treated with ranolazine than in the group given vehicle (Table). In addition, stroke volume was 36% higher in the ranolazine group. There was a non-significant trend toward lower end-systolic volume in the ranolazine group ($p = 0.15$). Overall, ejection fraction decreased with increasing necrosis, however ejection fraction tended to be higher in the ranolazine group regardless of infarct size (Fig 2). Independent of infarct size, ranolazine maintained ejection fraction significantly better then the vehicle ($p = 0.029$ by ANCOVA testing for group effect), suggesting that the drug benefited function in the stunned myocardium within the peri-infarct area.

Wall motion abnormality

After 3 hours of reperfusion, the primary wall motion abnormality was akinesis with a lesser extent of dyskinesis (Fig 3). In the vehicle group 0.34 ± 0.03 of the diastolic circumference was akinetic or dyskinetic, but in the ranolazine group wall motion abnormality was significantly smaller comprising 0.23± 0.03 of the circumference ($p = 0.02$).

Reflow to the jeopardized region

RMBF at the end of the reperfusion period was similar in both groups in the non-ischemic region (2.77 ± 0.39 ml/min/g ranolazine and 2.80 ± 0.30 ml/min/g in the vehicle group, p = ns). Reflow to the risk region was reduced in both groups. In the risk region, RMBF was 1.08 ± 0.20 ml/min/g in the ranolazine group and 0.91 ± 0.24 ml/min/g in the vehicle group (p = ns). Relative reflow to the risk region was highly correlated with necrosis in the two groups ($r = 0.82$, $p < 0.0001$) (Fig 4). However there was no significant group effect on this relationship. Thus overall the return of blood flow was related to the extent of necrosis with smaller infarcts having better reflow after reperfusion, but ranolazine did not alter reflow independently of reducing infarct size.

Discussion

In the present study, we examined the effects of pre-treatment with ranolazine on anatomic myocardial infarct size, LV dysfunction and return of blood flow after 60 minutes of ischemia and 3 hours of reperfusion. Our data show that ranolazine treatment reduced the extent of necrosis and improved LV function compared with the vehicle. Indices of global ventricular function such as ejection fraction and stroke volume were better in the ranolazine group compared with the vehicle group, and indices of regional function such as wall motion abnormality were also improved by ranolazine. Our observation that treatment with ranolazine maintained LV function better than vehicle for any extent of necrosis (in both small and large infarcts) suggests that ranolazine improved function not only by reducing necrosis but also by favorably affecting the peri-infarcted viable but stunned myocardium. Our data are consistent

with other studies in animals and humans in that ranolazine had no effect on heart rate or blood pressure, so the beneficial effects observed in the present study were independent of changes in oxygen consumption.

In our study of 60 minutes of ischemia followed by reperfusion, treatment with ranolazine reduced necrosis by 23% . Previous studies in our laboratory have tested interventions in rabbits subjected to between 30 and 120 minutes of ischemia followed by reperfusion. With 30 minutes of ischemia, treatment with a drug such as carporide (a Na+/H+ exchange inhibitor), for example, resulted in a reduction of infarct size of 55% compared with control (Hale and Kloner, 2000). With 120 minutes of ischemia, cooling of the heart reduced infarct size by 18% compared with normothermic hearts (Hale and Kloner, 1998).

Some previous studies have tested ranolazine in isolated heart preparations. McCormack and coworkers studied ranolazine in isolated, working rat hearts (McCormack et al., 1996). They found that under normoxic conditions, ranolazine treatment itself had no effect on baseline hemodynamic or contractile parameters. After 30 minutes of low-flow ischemia and reflow for one hour, indices of functional recovery such as cardiac work and rate/pressure product were better in ranolazine perfused hearts than in control hearts when treatment was initiated before the onset of ischemia.

In a model of global ischemia in Langendorff-perfused rabbit hearts, pre-treatment with ranolazine significantly reduced the release of CK and improved left ventricular developed pressure and dP/dt during reperfusion. Gralinski and coworkers also noted that the increase in tissue calcium seen in control hearts was completely prevented by 20μM ranolazine (Gralinski et al., 1994). In a guinea-pig heart model of low-flow ischemia, Clarke and coworkers (1993) found that hearts were perfused with ranolazine had less LDH and CK release during the ischemic period and tissue ATP was preserved.

Few studies have tested the effects of ranolazine on ischemic damage in intact animal models. Alley and Alps (1990) subjected baboons to 30 minutes of coronary artery occlusion

followed by 5.5 hours of reperfusion. Ranolazine (500 µg/kg bolus and 50 µg/kg/minute infusion) was given 10 minutes before occlusion in treated animals. Myocardial enzyme release was used as a marker of ischemic damage. Compared with control animals, total creatine kinase and lactic dehydrogenase release during the reperfusion period was significantly lower in the ranolazine group. Serum levels of CK_{MB} were 8 fold higher in the control group than in the ranolazine group at the end of the reperfusion period.

Zacharowski et al. (2001) tested the effects of a 10 mg/kg bolus dose and 9.6 mg/kg/hr infusion of ranolazine on infarct size and cardiac troponin T release in anesthetized, open-chest rats subjected to a 25-minute coronary artery occlusion and two hours of reperfusion. This study showed that ranolazine treatment reduced infarct size in rats by about 33% and significantly reduced troponin release.

Black and coworkers tested ranolazine in a canine model of 90-minute coronary artery occlusion and 18 hours of reperfusion (Black et al., 1994). Treatment (3.3 mg/kg for 2 minutes and 7.2 mg/kg/hr) was initiated 30 minutes before onset of ischemia. In contrast with other studies, no significant differences were noted in CK release or infarct size. This discrepancy might be related to differences in species tested or to the long duration of ischemia in their study.

As an investigational drug, ranolazine was shown in several clinical trials to reduce the pain frequency of angina and to prolong exercise time in patients with coronary artery disease (Jain et al., 1990; Thadani et al., 1994; Rosseau et al., 2005; Pepine et al., 1999; Chaitman et al., 2004a; Chaitman et al., 2004b). It has been shown to be safe and effective used alone (Chaitman et al., 2004a), or in combination with other agents used in the treatment of angina (Chaitman et al., 2004b). Ranolazine has recently been approved by the FDA for use in patient with chronic angina who do not to other conventional angina therapies.

In contrast with other anti-anginal treatments that work by decreasing indices of cardiac work, ranolazine does not affect heart rate or blood pressure, suggesting a different mode of action. The precise mechanism of action for ranolazine's benefit in the setting of myocardial

ischemia remains under investigation. An early theory was that ranolazine functions to partially inhibit fatty acid oxidation, shifting metabolism during ischemia toward glucose oxidation with increased efficiency of oxygen use (McCormack et al., 1996). However a more recent study found that ranolazine improved post-ischemic cardiac function at a concentration (20μM) that causes no decrease in fatty acid oxidation. In this latter study, 100μM ranolazine, a concentration that is 10 to 20-fold higher than the therapeutic dose, inhibited fatty acid oxidation by only 12% (MacInnes et al., 2003). New research provides evidence that the cardioprotective actions of ranolazine are related to its effect of inhibiting the late sodium channel in cardiac cells (Song et al., 2004;Antzelevitch et al., 2004; MacInnes et al., 2003). The late component of the sodium current can be increased by ischemic metabolites (Undrovinas et al., 1992;Wu and Corr, 1994) and by oxygen free radicals released at reperfusion (Ward and Giles, 1997). Intracellular sodium is then exchanged for intracellular calcium via the sodium-calcium exchanger. Calcium overload in myocytes then causes mechanical dysfunction and cell death. Regardless of the mechanism of action of ranolazine, drugs that reduce calcium influx during ischemia/reperfusion are expected to be cardioprotective.

The relative contribution of the late I_{Na} to the rise in $[Na^+]_i$ during ischemia appears to depend on the experimental conditions (Bers et al., 2003; Xiao and Allan, 1999). In these studies the increase in $[Na^+]_i$ during ischemia was in great part due to entry of Na^+ through the persistent Na^+ channels, i.e., late I_{Na}. On the other hand, Murphy et al., 1999 proposed that both the Na^+/H^+ exchanger and the non-inactivating Na^+-channels are major contributors of the rise in $[Na^+]_i$.

We tested only one dose (concentration of ranolazine) in the present study. This dose of ranolazine was based on therapeutic levels for angina treatment in humans and may not have provided maximal efficacy in our model. Based on results of other studies describing the cardioprotective effects of ranolazine in isolated perfused hearts (Gralinski et al. 1994, Clarke et al. 1993), concentrations of 15 and 20 μM of ranolazine were found to be more efficacious than

5 and 10 μM, concentrations similar to that achieved in our study. In the present study, the dose used yielded plasma concentrations within the range of clinical therapeutic plasma levels, as was our intention. However we cannot exclude the possibility that a high dose may have provided enhanced protection.

Our aim in the present study was to test the effects of ranolazine treatment on anatomic infarct size expressed as a function of the ischemic risk zone and to evaluate its effects on global and regional LV function. Our study is the first that we are aware of showing that ranolazine both decreased anatomic infarct size and caused an improvement in LV function after reperfusion in an in vivo rabbit model. We demonstrated not only improved global LV function but improved regional LV wall function as well. Our findings show that ranolazine provides these benefits, both reducing necrosis and improving cardiac function, without altering heart rate or blood pressure, unlike other antianginal agents.

References

Allely MC and Alps BJ (1990) Prevention of myocardial enzyme release by ranolazine in a primate model of ischaemia with reperfusion. Br J Pharmacol **99**:5-6.

American Heart Association. Position of the American Heart Association on research animal use (1985) Circulation **71**:849A.

Antzelevitch C, Belardinelli L, Zygmunt AC, Burashnikov A, Di Diego JM, Fish JM, Cordeiro JM and Thomas G (2004) Electrophysiological effects of ranolazine, a novel antianginal agent with antiarrhythmic properties. Circulation **110**:904-910.

Belardinelli L, Antzelevitch C and Fraser H (2004) Inhibition of late (sustained/persistent) sodium channel: a potential drug target to reduce intracellular sodium-dependent calcium overload and its detrimental effects on cardiomyocyte function. Eur Heart J **6**: [Suppl 1] 13-17.

Belardinelli L, Shryock JC and Fraser H (2006) The mechanism of ranolazine action to reduce ischemia-induced diastolic dysfunction. Eur Heart J **8**[Suppl A]:A10-A13.

Bers DM, Barry WH and Despa S (2003)Intracellular Na+ regulation in cardiac myocytes. Cardiovasc Res **57**:871-872.

Black SC, Gralinski MR, McCormack JG, Driscoll EM and Lucchesi BR (1994) Effect of ranolazine on infarct size in a canine model of regional myocardial ischemia/reperfusion. J Cardiovasc Pharmacol **24**:921-928.

Chaitman BR, Skettino SL, Parker JO, Hanley P, Meluzin J, Kuch J, Pepine CJ, Wang W, Nelson JJ, Herbert DA and Wolffe, AA for the MARISA Investigators (2004a) Anti-ischemic effects and long-term survival during ranolazine monotherapy in patients with chronic severe angina. JACC **43**:1375-1382.

Chaitman BR, Pepine CJ, Parker JO, Skopal J, Chumakova G, Kuch J, Wang W, Skettino SL and Wolff AA; Combination Assessment of Ranolazine In Stable Angina (CARISA) Investigators (2004b) Effects of ranolazine with atenolol, amlodipine, or diltiazem on exercise tolerance and angina frequency in patients with severe chronic angina. A randomized controlled trial. JAMA **291**:309-316.

Clarke B, Spedding M, Patmore L and McCormack JG (1993) Protective effects of ranolazine in guinea-pig hearts during low-flow ischaemia and their association with increases in pyruvate dehydrogenase. Br J Pharmacol **109**:748-750.

Fredj S, Sampson KJ, Liu H and Kass, RS (2006) Molecular basis of ranolazine block of LQT-3 mutant sodium channels: evidence for site of action. Br J Pharmacol Mar 6; [Epub ahead of print]

Gralinski MR, Black SC, Kilgore KS, Chou AY, McCormack JG and Lucchesi BR (1994) Cardioprotective effects of ranolazine (RS-43285) in the isolated perfused rabbit heart. Cardiovasc Res **28**:1231-1237.

Hale SL and Kloner RA (1998) Myocardial temperature reduction attenuates necrosis after prolonged ischemia in rabbits. Cardiovasc Res **40**:502-507.

Hale SL and Kloner RA (2000) Effect of combined KATP channel activation and Na+/H+ exchange inhibition on infarct size in rabbits. Am J Physiol **279**:H2673-H2677.

Imahashi K, Kusuoka H, Hashimoto K, Yoshioka J, Yamaguchi H and Nishimura T (1999) Intracellular sodium accumulation during ischemia as the substrate for reperfusion injury. Circ Res **84**:1401-1406.

Jain D, Dasgupta P, Hughes LO, Lahiri A and Raftery EB (1990) Ranolazine (RS-43285): A preliminary study of a new anti-anginal agent with selective effect on ischaemic myocardium. Eur J Clin Pharmacol **38**:111-114.

MacInnes A, Fairman DA, Binding P, Rhodes J, Wyatt MJ, Phelan A, Haddock PS and Karran EH (2003) The antianginal agent trimetazidine does not exert its functional benefit via inhibition of mitochondrial long-chain 3-ketoacyl coenzyme A thiolase. Circ Res **93**:26-32.

McCormack JG, Barr RL, Wolff AA and Lopaschuk GD (1996) Ranolazine stimulates glucose oxidation in normoxic, ischemic and reperfused ischemic rat hearts. Circulation **93**:135-142.

Murphy E, Cross H and Steenbergen C (1999) Sodium regulation during ischemia versus reperfusion and its role in injury. Circ Res **84**:1469-1470.

Pepine CJ and Wolffe AA. on behalf of the Ranolazine group (1999) A controlled trial with a novel anti-ischemic agent, ranolazine, in chronic stable angina pectoris that is responsive to conventional antianginal agents. Am J Cardiol **84**:46-50.

Rousseau MF, Pouleur H, Cocco G and Wolff AA (2005) Comparative efficacy of ranolazine versus atenolol for chronic angina pectoris. Am J Cardiol **95**:311-316.

Song Y, Shryock JC, Wu L and Belardinelli L (2004) Antagonism by ranolazine of the pro-arrhythmic effects of increasing late I_{NA} in guinea pig ventricular myocytes. J Cardiovasc Pharmacol **44**:192-199.

Thadani U, Ezekowitz M, Fenney L and Chiang Y-K for the Ranolazine group (1994) Double-blind efficacy and safety study of a novel anti-ischemic agent, ranolazine, versus placebo in patients with chronic stable angina. Circulation **90**:726-734.

Undrovinas AI, Fleidervish IA and Makielski JC (1992) Inward sodium current at resting potentials in single cardiac myocytes induced by the ischemic metabolite lysophosphatidylcholine. Circ Res **71**:1231-1241.

Undrovinas A, Belardinelli L, Undrovinas N, and Sabbah H (2006) Ranolazine improves abnormal repolarization and contraction in left ventricular myocytes of dogs with heart failure by inhibiting late sodium current. J Cardiovasc Electrophysiol **17** (Suppl. 1):S1-S9.

Ward CA and Giles WR (1997) Ionic mechanism of the effects of hydrogen peroxide in rat ventricular myocytes. J Physiol **500**:631-642.

Wu J and Corr PB (1994) Palmitoyl carnitine modifies sodium currents and induces transient inward current in ventricular myocytes. Am J Physiol **266**:H1034-H1046.

Xiao XH and Allen DG (1999)Role of Na(+)/H(+) exchanger during ischemia and preconditioning in the isolated rat heart. Circ Res **85**:723-730.

Zacharowski K, Blackburn B and Thiemermann C (2001) Ranolazine, a partial fatty acid oxidation inhibitor, reduced myocardial infarct size and cardiac troponin T release in the rat. Eur J Pharmacol **418**:105-110.

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Treatment drug (ranolazine) and vehicle were supplied by CV Therapeutics in vials whose contents were blinded to the investigator. The authors had no affiliation with CV Therapeutics and no financial conflict of interest.

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Figure Legends

Figure 1

There were no significant differences between the two groups in hemodynamics or contractility over the duration of the study.

Figure 2

Relationship between ejection fraction measured by left ventriculography and the extent of necrosis expressed as a percent of the LV. Regardless of infarct size, ranolazine maintained ejection fraction significantly better then the vehicle ($p = 0.029$ by ANCOVA testing for group effect).

Figure 3

The left ventriculogram was analyzed and areas of akinesis and dyskinesis measured and expressed as a percent of the diastolic circumference. Wall motion abnormality (percent akinetic and percent both akinetic plus dyskinetic) was significantly less in the ranolazine treated group. * $p < 0.05$.

Figure 4

Relative blood flow (RMBF in the risk zone/ RMBF in the non-ischemic zone) at the end of the reperfusion period was significantly correlated with infarct size as a percent of the left ventricle. There was no group effect on the relationship suggesting that ranolazine did not alter reperfusion blood flow independently of reducing infarct size.

Table: Indices of Left Ventricular Function at the End of Reperfusion Assessed by Left Ventriculogram

	Ranolazine	Vehicle	P =
End-diastolic volume (ml)	2.47 ± 0.09	2.34 ± 0.09	0.30
End-systolic volume (ml)	1.42 ± 0.06	1.56 ± 0.08	0.15
Ejection fraction	0.42 ± 0.02	0.33 ± 0.02	**0.007**
Stroke volume (ml)	1.05 ± 0.08	0.78 ± 0.07	**0.013**

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Figure 1

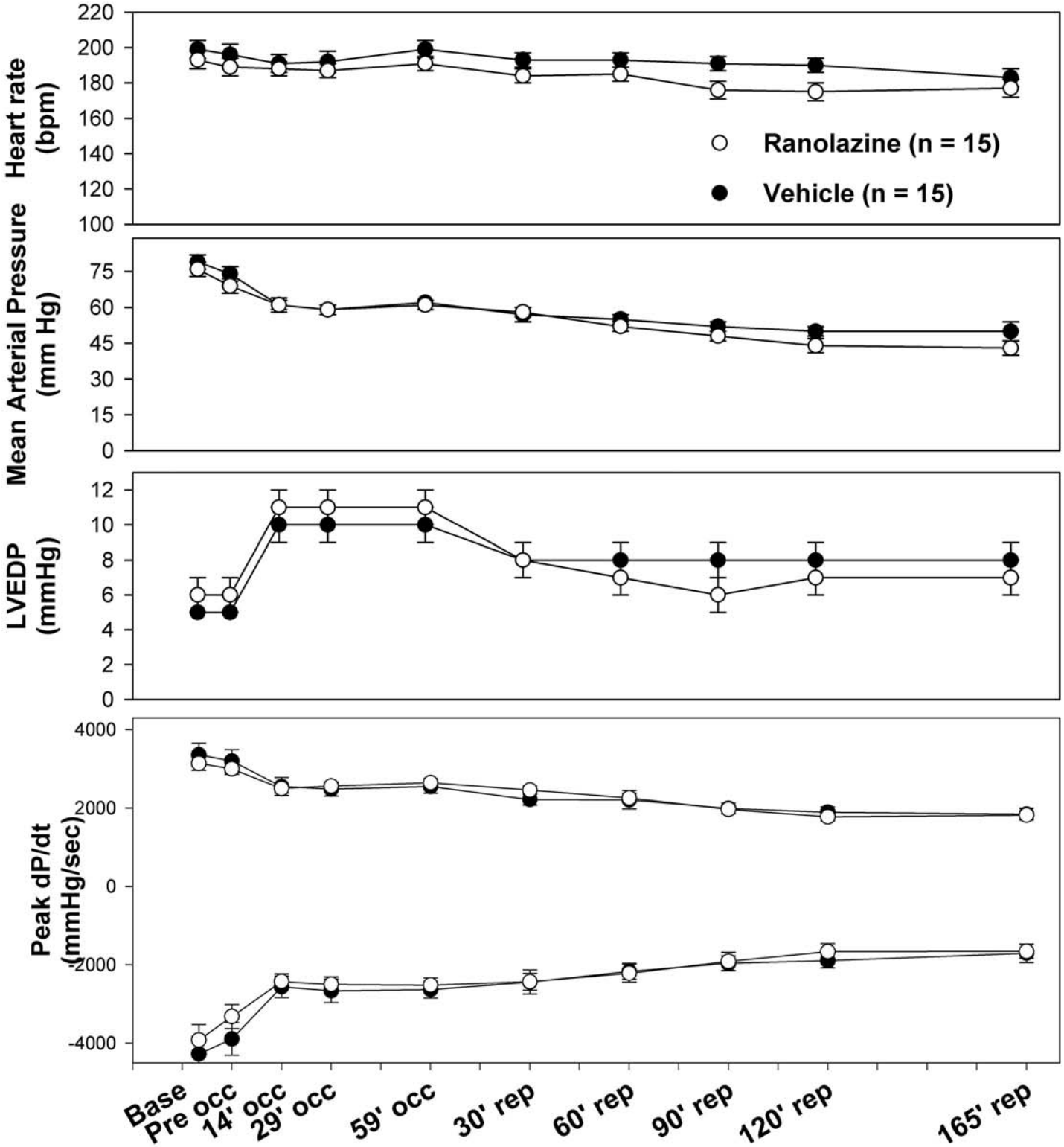

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Figure 2

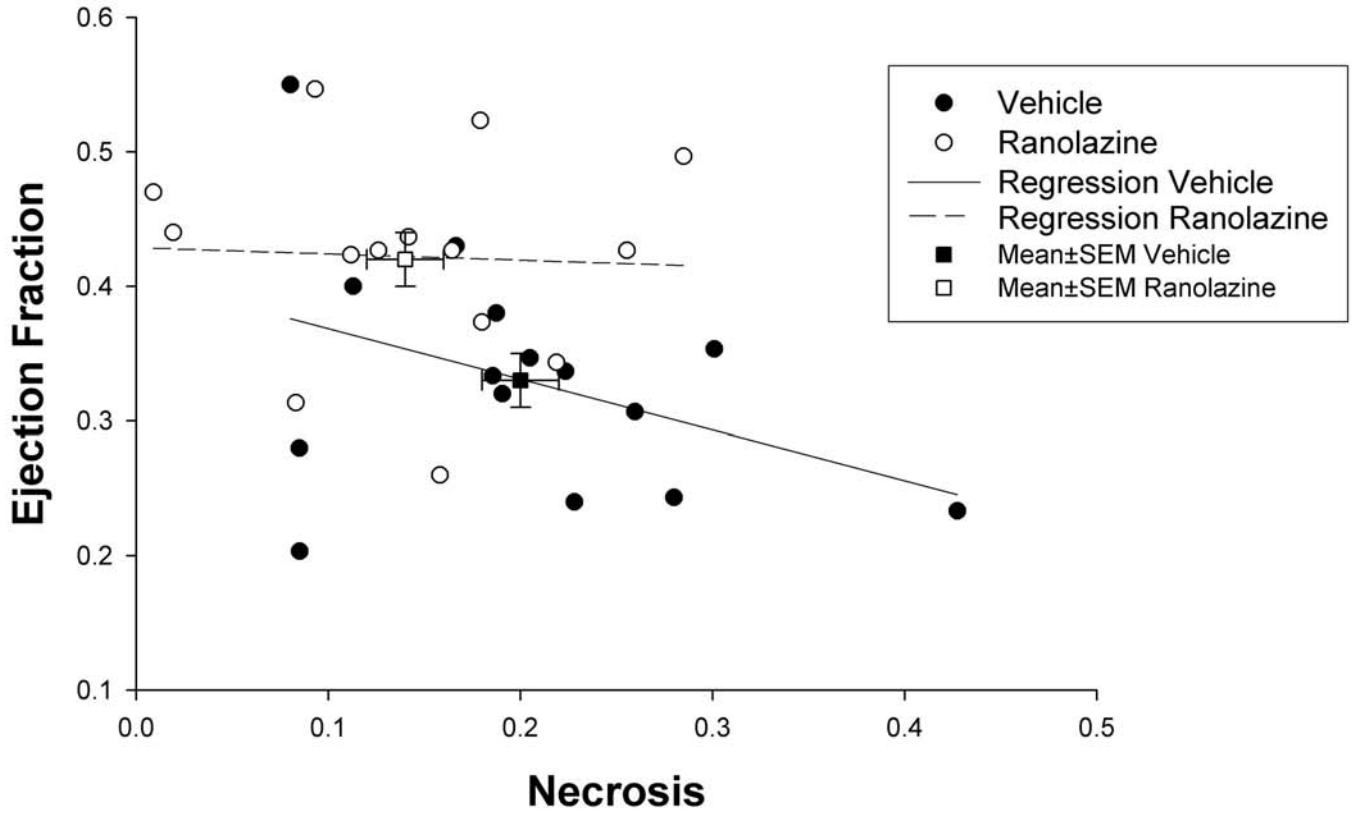

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Figure 3

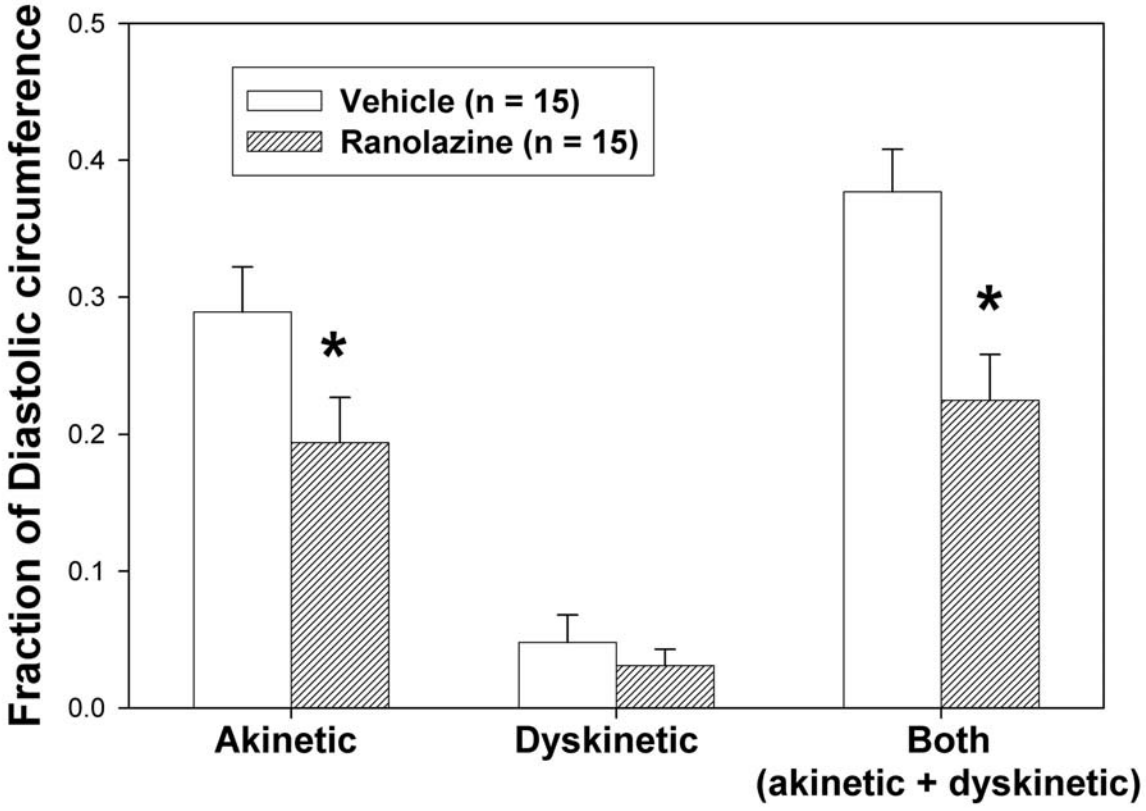

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Figure 4