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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ABSTRACT

Ranolazine is an inhibitor of the late sodium current 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 h of 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 versus 0.33 ± 0.02; p < 0.007) and stroke volume (1.05 ± 0.08 versus 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 ± 0.03 versus 0.34 ± 0.03 in vehicle-treated group; p < 0.02. The ischemic risk region was similar in both groups; however, infarct size was significantly smaller in the treated group (44 ± 5 versus 57 ± 4% vehicle; p < 0.04). There were no significant differences among groups in heart rate, arterial pressure, LV end-diastolic pressure, or maximum-positive or -negative first time derivative of LV pressure (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.
(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 current can lead to subsequent intracellular calcium overload via the Na+/Ca2+ exchanger. Calcium overload in myocytes then causes mechanical dysfunction and may contribute to 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 current, 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; Rousseau et al., 2005; Pepine and Wolfe, 1999; Chaitman et al., 2004a,b); it has been shown to be safe and effective when used alone (Chaitman et al., 2004b) or in combination with other agents in the treatment of angina (Chaitman et al., 2004a). Ranolazine has recently been approved by the United States Food and Drug Administration for treatment of chronic angina in patients who fail to respond to other angina drugs. In contrast with other antianginal 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 creatine kinase (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.

Materials and Methods

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 accredited 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 was exposed. Near the base of the heart, the first large anterolateral branch of the circumflex artery or the circumflex artery itself was encircled with a 4-0 silk suture. Coronary occlusion in this region normally results in ischemia of a large territory of the anterolateral 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 five rabbits after a bolus dose of ranolazine, 2 mg/kg injected over 60 s, 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 min from giving the bolus dose.

Experimental Protocol. After surgical preparation and a 15-min stabilization period, baseline hemodynamic parameters and temperature were obtained. The rabbits were randomized to receive ranolazine (2 mg/kg bolus, injected over 60 s, plus 60 μg/kg/min; n = 15) or an equivalent amount of vehicle (n = 15); the investigator was not knowledgeable of the treatment until the completion of the entire study. Treatment was initiated 10 min 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 min of coronary artery occlusion followed by 3 h of reperfusion. Hemodynamic parameters were monitored and recorded at baseline before CAO, at 15, 29, and 59 min of occlusion, and at 30, 60, 90, 120, and 165 min 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 reoccluded, 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. Heart rate, LV systolic pressure, LV end-diastolic pressure (LVEDP), and maximum and negative first time-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 1 Ks using an Advanced Digital Instruments system (Grand Junction, CO). 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 milliliters of contrast medium were injected into the left ventricle, and the image of the left ventricular cavity was recorded on videotape. Later measurements of end-systolic and end-diastolic volumes, ejection fraction, and stroke volume were measured in three consecutive beats, and the results were 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. Regional myocardial blood flow (RMBF) was measured using approximately 2 × 10⁶ radioactive microspheres (15μ; Perkin Elmer Life Sciences, Boston, MA) labeled with ¹⁴Cce or ¹⁰³Ru. Microspheres were injected into the left atrium through a left atrial catheter, and that was 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 nonischemic regions. The samples were weighed and counted together with the reference blood samples in a computerized gamma well counter (System S100; Canberra Industries, Meriden, CT). RMBF was computed, and the results were expressed as milliliter/minute/gram. 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 nonischemic zone.

Analysis of Risk Zone and Necrosis. The heart was sliced transversely into six to eight 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 min, immersed in formalin, and rephotographed. 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 percentage of the risk zone that was necrotic.

Statistical Analyses. Data were tabulated and calculated using Excel worksheets. All statistical analyses were performed using SAS, version 6.04 (SAS Institute, 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 ± S.E.M.

Results

Ranolazine Plasma Levels. Before the present study, the dosing regimen and blood levels of ranolazine were studied in five rabbits. At 5 min 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 ± 0.5 µM). Over the time period of the study (240 min), ranolazine concentrations in the five animals ranged between an average of 4.5 ± 0.5 and 8.8 ± 0.5 µM. These blood levels are comparable with the therapeutic range in humans (Chaitman et al., 2004b).

Hemodynamics. No significant differences among 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 percentage 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 percentage 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 1). 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 than 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 h 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 signif-

| TABLE 1 |
| Indices of left ventricular function at the end of reperfusion assessed by left ventriculogram |
| Ranolazine | Vehicle | P Value |
| 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 |
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 nonischemic region (2.77 ± 0.39 ml/min/g ranolazine and 2.80 ± 0.30 ml/min/g in the vehicle group; \(P = \text{NS}\)). 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 (\(P = \text{NS}\)). 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 pretreatment with ranolazine on anatomic myocardial infarct size, LV dysfunction, and return of blood flow after 60 min of ischemia and 3 h 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 that show that ranolazine had no effect on heart rate or blood pressure; thus, the beneficial effects observed in the present study were independent of changes in oxygen consumption.

In our study of 60 min of ischemia followed by reperfusion, treatment with ranolazine reduced necrosis by 23%. Previous studies in our laboratory have tested interventions in rabbits subjected to 30 and 120 min of ischemia followed by reperfusion. With 30 min 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 min 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 et al. (1996) studied ranolazine in isolated working rat hearts. They found that, under normoxic conditions, ranolazine treatment itself had no effect on baseline hemodynamic or contractile parameters. After 30 min of low-flow ischemia and reflow for 1 h, 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, pretreatment with ranolazine significantly re-
duced the release of CK and improved left ventricular developed pressure and dP/dt during reperfusion. Gralinski et al. (1994) noted that the increase in tissue calcium seen in control hearts was completely prevented by 20 μM ranolazine. In a guinea pig heart model of low-flow ischemia, Clarke et al. (1993) found that hearts perfused with ranolazine had less LDH and CK release during the ischemic period and that 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 min of coronary artery occlusion followed by 5.5 h of reperfusion. Ranolazine (500 µg/kg bolus and 50 µg/kg/min infusion) was given 10 min 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 CKMB were 8-fold higher in the reperfusion period was significantly lower in the ranolazine group. Serum levels of CKMB were 8-fold higher in the control group than in the ranolazine group at the end of the reperfusion period.

Zacharako et al. (2001) tested the effects of a 10 mg/kg bolus dose and 9.6 mg/kg/h infusion of ranolazine on infarct size and cardiac troponin T release in anesthetized, open-chest rats subjected to a 25-min coronary artery occlusion and 2 h of reperfusion. This study showed that ranolazine treatment reduced infarct size in rats by approximately 33% and significantly reduced troponin release.

Black et al. (1994) tested ranolazine in a canine model of 90-min coronary artery occlusion and 18 h of reperfusion. Treatment (3.3 mg/kg for 2 min and 7.2 mg/kg/h) was initiated 30 min 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; Rousseau et al., 2005; Pepine and Wolffe, 1999; Chaitman et al., 2004a, b). It has been shown to be safe and effective when used alone (Chaitman et al., 2004b) or in combination with other agents used in the treatment of angina (Chaitman et al., 2004a). Ranolazine has recently been approved by the United States Food and Drug Administration for patients with chronic angina who do not use other conventional angina therapies.

In contrast with other antianginal 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 the benefit of ranolazine 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 postischemic cardiac function at a concentration (20 μM) that causes no decrease in fatty acid oxidation. In this latter study, 100 μM 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 current in cardiac cells (MacInnes et al., 2003; Antzelevitch et al., 2004; Song et al., 2004). 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 INa, to the rise in [Na+]i, during ischemia seems to depend on the experimental conditions (Xiao and Allen, 1999; Bers et al., 2003). 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 INa. On the other hand, Murphy et al. (1999) proposed that both the Na+/H+ exchanger and the noninactivating 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 (Clarke et al., 1993; Gralinski et al., 1994), concentrations of 15 and 20 μM 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 higher 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 also improved regional LV wall function. 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


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Ranolazine and Acute Myocardial Infarction


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