Ranolazine Increases β-Cell Survival and Improves Glucose Homeostasis in Low-Dose Streptozotocin-Induced Diabetes in Mice

Yun Ning, Wei Zhen, Zhuo Fu, Jenny Jiang, Dongmin Liu, Luiz Belardinelli, and Arvinder K. Dhalla

Department of Biology, Gilead Sciences, Palo Alto, California (Y.N., J.J, L.B., A.K.D.); and Department of Human Nutrition, Foods and Exercise, Virginia Polytechnic Institute and State University, Blacksburg, Virginia (W.Z., Z.F., D.L.)

Received October 22, 2010; accepted January 10, 2011

ABSTRACT

In addition to its anti-ischemic and antianginal effects, ranolazine has been shown to lower hemoglobin A1c (HbA1c) in patients with coronary artery disease and diabetes. The present study was undertaken to test the hypothesis that ranolazine lowers HbA1c because of improved glucose homeostasis in an animal model. Diabetes in mice was induced by giving multiple low doses of streptozotocin. Ranolazine was given twice daily via an oral gavage (20 mg/kg) for 8 weeks. Fasting plasma glucose levels were significantly lower in the ranolazine-treated group (187 ± 19 mg/dl) compared with the vehicle group (273 ± 23 mg/dl) at 8 weeks. HbA1c was 5.8 ± 0.4% in the vehicle group and 4.5 ± 0.2% in the ranolazine-treated group (p < 0.05). Glucose disposal during the oral glucose tolerance test (OGTT) and insulin tolerance test were not different between the two groups; however, during OGTT, peak insulin levels were significantly (p < 0.05) higher in ranolazine-treated mice. Mice treated with ranolazine had healthier islet morphology and significantly (p < 0.01) higher β-cell mass (69 ± 2% per islet) than the vehicle group (50 ± 5% per islet) as determined from hematoxylin and eosin staining. The number of apoptotic cells was significantly (p < 0.05) less in the pancreas of the ranolazine-treated group (14 ± 2% per islet) compared with the vehicle group (24 ± 4% per islet). In addition, ranolazine increased glucose-stimulated insulin secretion in rat and human islets in a glucose-dependent manner. These data suggest that ranolazine may be a novel antidiabetic agent that causes β-cell preservation and enhances insulin secretion in a glucose-dependent manner in diabetic mice.

Introduction

Type 2 diabetes is a major public health problem affecting more than 250 million people worldwide. The underlying causes of type 2 diabetes are a combination of impairment in insulin-mediated glucose disposal (insulin resistance) and a defect in insulin secretion from pancreatic β-cells (Wajchenberg, 2007). Type 2 diabetes is also a risk factor for cardiovascular disease and frequently coexists with cardiovascular comorbidities, including dyslipidemia and hypertension, and thus is a significant predictor of cardiovascular mortality (Cannon, 2008). It is reported that the presence of diabetes in patients with coronary artery disease (CAD) doubles their risk for adverse cardiovascular events (Peippe et al., 2003; Bakris et al., 2004). The treatment options for diabetic patients with CAD currently have several safety concerns. Several clinical trials have shown that patients with CAD treated with β-blockers and calcium channel blockers have consistently and significantly higher rates of newly diagnosed diabetes because of the prodiabetic effects of these medications (Peippe and Cooper-Dehoff, 2004; Wofford and King, 2004; Dahloff et al., 2005).

Ranolazine is a novel anti-ischemic and antianginal drug that reduces angina frequency and improves exercise performance in patients with chronic stable angina, an insidious manifestation of CAD (Chaitman et al., 2004; Stone et al., 2006). Ranolazine reduces myocardial ischemia by improving sodium-calcium homeostasis via inhibition of the late phase of the inward sodium current (late INa) during cardiac repolarization. In addition to its antianginal effects, ranolazine has been shown to reduce HbA1c in patients with CAD and diabetes in two clinical studies (Timmis et al., 2006; Scirica et al., 2007; Chisholm et al., 2010). In the CARISA (Combina-
tion Assessment of Ranolazine In Stable Angina) trial (Timmis et al., 2006), ranolazine treatment for 12 weeks significantly reduced HbA1c levels by 0.7 ± 0.18% regardless of concomitant insulin and/or oral hypoglycemic therapy. In the MERLIN-TIMI 36 (Metabolic Efficiency with Ranolazine for Less Ischemia in Non—ST-Elevation Acute Coronary Syndrome Thrombolysis in Myocardial Infarction 36) trial, a 0.6 ± 0.2% reduction in HbA1c was observed in patients treated with ranolazine in addition to standard antidiabetic therapy. Furthermore, in patients with moderate/severe hyperglycemia, ranolazine treatment was associated with a reduction (25.7 mg/dl) in fasting plasma glucose (FPG) after 4 months of treatment (Scirica et al., 2007; Chisholm et al., 2010). However, no preclinical data have been reported, demonstrating either the antidiabetic properties of ranolazine or the pharmacological basis for reduction of HbA1c observed in clinical trials.

Therefore, the objectives of the present study were 1) to reproduce the clinical findings with ranolazine in an animal model, 2) to correlate the HbA1c-lowering effect with glucose levels, and 3) to understand the mechanisms underlying the antidiabetic effects of ranolazine. We determined the effects of an 8-week treatment with ranolazine in a diabetic mouse model induced with multiple low doses of streptozotocin. We monitored body weight and FPG weekly and HbA1c every 4 weeks during 8 weeks of ranolazine treatment followed by oral glucose tolerance test (OGTT) and insulin tolerance test at the end of the treatment. Pancreatic islet β-cell mass and apoptosis rate were also determined. Our data show that long-term treatment with ranolazine in STZ-induced diabetic mice improves FPG and HbA1c levels and the insulin response during an OGTT. Moreover, STZ mice treated with ranolazine had healthier islet morphology and significantly higher β-cell mass compared with the vehicle-treated group. The number of apoptotic islet cells was also significantly less in pancreas from ranolazine-treated mice. In addition, we investigated the acute effect of ranolazine on GSIS in isolated islets. Our data show that ranolazine increases GSIS in both rat and human-pancreatic islets in a concentration- and glucose-dependent manner. Together, these data suggest that long-term treatment with ranolazine results in β-cell preservation and enhances insulin secretion, which could explain the improvement in glucose and HbA1c levels in diabetic mice.

Materials and Methods

Animals. All experimental procedures were performed under a protocol approved by the Institutional Animal Care and Use Committee of Gilead Palo Alto Inc. (Palo Alto) and in accordance with the recommendations set forth in the Guide for the Care and Use of Laboratory Animals published by the National Research Council. Five-week-old male C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were housed five per cage in a room maintained on a 12-h light/dark cycle (lights on from 6:00 AM to 6:00 PM) under constant temperature (22–25°C) with access to food and water ad libitum.

Generation of Multiple Low-Doses of Streptozotocin Diabetic Animal Model. After an initial acclimation period (7 days), mice were injected with streptozotocin (40 mg/kg i.p., dissolved in ice-cold 0.025 M sodium citrate-buffered solution, pH 4.5, freshly made right before injection) for 5 consecutive days. Fasting blood glucose levels were monitored 2 days after STZ treatment. The diabetic mice were then divided into STZ + vehicle and STZ + ranolazine groups (10 mice/group) based on body weight and blood glucose levels. Age and sex-matched non-diabetic mice were used as “normal” controls (n = 3). For the next 8 weeks, mice were given either vehicle or ranolazine (20 mg/kg in water p.o., twice daily). Plasma concentrations of ranolazine after an oral dose of 20 mg/kg in mice at 15, 30, and 60 min were determined to be 9.1 ± 3.3, 7.4 ± 3.0, and 6.1 ± 2.8 μM, respectively, which are within the clinically therapeutic concentrations (5–10 μM) of the drug. Body weight and fasting blood glucose levels were monitored once a week. HbA1c levels were measured using a DCA 2000+ clinical analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) at 0, 4, and 8 weeks of treatment. At the end of 8-week treatment, the OGTT and insulin tolerance test were performed.

Oral Glucose Tolerance Test. Mice were fasted for 4 h (8:00 AM–12:00 PM) before the OGTT. At 0 min, a drop of blood (5 μl) was taken via a tail nick before giving the oral glucose load. Mice were then given a glucose load (2 g/kg) in distilled water. Blood samples (40 μl) were obtained at 15, 30, 60, 90, 120, 180, and 240 min after glucose load for the determination of blood glucose with a glucometer. Insulin levels were measured using a rat/mouse insulin enzyme-linked immunosorbent assay kit for each sample. At the end of the experiment, the animals were euthanized, and the pancreases from all mice were collected for morphological and immunohistochemical analysis.

Insulin Tolerance Test. Mice were fasted for 4 h (8:00 AM–12:00 PM) and then given an intraperitoneal injection of insulin (1 U/kg) in distilled water. Blood samples were taken at 0, 15, 30, 60, 90, and 120 min. Blood glucose levels were determined using a glucometer (Roche Diagnostics, IN). Islet Morphometry. Pancreases were fixed in 10% formalin overnight and then embedded in paraffin. Sections (7 μm) were obtained throughout the pancreas and were deparaffinized and rehydrated sequentially in xylene, xylene/ethanol, and ethanol (100, 95, 80, 70, and 50%), and then the sections were placed in distilled water for 10 min. Pancreatic sections were stained with hematoxylin and eosin (H&E) using standard protocols.

Immunohistochemistry. Pancreas from age and sex-matched mice were stained as normal control. A series of pancreatic sections (7 μm thickness, n > 4) from normal, STZ + vehicle, and STZ + ranolazine groups were prepared, mounted on glass slides, and stained for insulin, glucagon, and apoptosis using fluorescent antibodies. In brief, the tissue sections were blocked with 10% donkey serum in PBS buffer for 30 min and then washed with PBS. For the detection of insulin and glucagon, a cocktail of primary antibodies (guinea pig anti-insulin, mouse antigliucagon) was added to the slides and incubated at 4°C overnight. Slides were washed with PBS and then incubated with a cocktail of secondary antibodies to the respective primaries (donkey anti-guinea pig Cy3, donkey anti-mouse FITC) for 30 min at room temperature. Staining was preserved, and nuclei were identified by adding a drop of Vectashield mounting medium (Vector Laboratories, Burlingame, CA) with 4',6-diamidino-2-phenylindole to each tissue section before placing coverslip. Using this procedure, insulin is stained red, whereas glucagon appears green and nuclei are stained blue. Similar procedure was used to detect apoptotic β-cells as determined by sequential labeling of the sections with activated caspase-3 antibody and donkey anti-rabbit FITC secondary antibody. All sections were viewed under fluorescent microscope, and the stained areas were digitally photographed at a magnification of 20×. The images taken at different magnification were normalized using the standard ruler grade (S1 Finder Graticule, 68040; Electron Microscopy Science, Hatfield, PA). Analyses of islet area and entire section areas were performed using ImageJ software (National Institutes of Health, Bethesda, MD). At least four sections from each animal (n = 3 mice in normal group; n = 6 mice for STZ + vehicle and STZ + ranolazine groups) were analyzed.

Isolation and Culture of Pancreatic Islets. Pancreatic islets were isolated from 8- to 12-week-old rats as described previously.
NaHCO3, 0.1% bovine serum albumin, 10 mM HEPES, pH 7.4) to made in dimethyl sulfoxide and immediately frozen at -80°C (Sigma-Aldrich, St. Louis, MO) gradient. Human islets were obtained through the National Institutes of Health-supported Islet Cell Resource Centers and the Islet Distribution Program at the Juvenile Diabetes Research Foundation, both of which are administered by Administrative and Bioinformatics Coordinating Center. The islet purity was 80 to 90%, and viability was 80 to 97%. Before the experiment, the islets were maintained in CMRL-1066 medium (Mediatech, Manassas, VA) containing 10% FBS (HyClone Laboratories, Logan, UT).

**Insulin Secretion.** Isolated pancreatic islets were maintained in complete RPMI 1640 medium overnight (Yang et al., 2004). Insulin secretion assay was performed essentially as described previously (Liu et al., 2006). In brief, before the experiment, islets were preincubated in Krebs-Ringer-bicarbonate (KRB) buffer containing 3 mM glucose for 30 min, after which islets were washed and incubated in triplicate in 24-well plate (50 islets/well), in oxygenated KRB buffer with 3 or 20 mM glucose in the presence of various concentrations of ranolazine or vehicle for 60 min at 37°C. In some experiments, islets were also incubated with glucagon-like peptide-1 (GLP-1) (Sigma-Aldrich), which served as a positive control. Insulin in experimental samples was measured by an enzyme-linked immunosorbent assay kit (Merodia, Winston-Salem, NC). Our preliminary experiments show that exposure of the islets to the compounds for 1 h had no effect on total insulin or protein content. Therefore, all insulin secretion data in the present study are presented as insulin secretion per well.

**Chemicals and Biological Reagents.** Ranolazine is a piperazine derivative chemically described as 1-piperazineacetamide [N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-, (±)]. Ranolazine was synthesized at Gilead Palo Alto Inc. For in vivo study, ranolazine was dissolved in 0.025 N HCl, pH 4.0, and the solution was filtered with a 0.2 μM sterilizing filter (Millipore, Billerica, MA). Other agents were purchased from the following sources: streptozotocin (S0130), human insulin (19278, 275 U/ml), glucose (G-7528), and GLP-1 (G9416; Sigma-Aldrich); rat/mouse insulin enzyme-linked immunosorbent assay kit (EZRMI-13K, Millipore); Accu-Chek active diabetic test strips and glucometer (Roche Diagnostics); and HbA1c measure system (5035C, DCA systems; Siemens Healthcare Diagnostics). The antibodies were purchased from following sources: 10% donkey serum (Jackson ImmunoResearch Laboratories, West Grove, PA), guinea pig anti-insulin (Sigma I-8510, 1:500; Sigma-Aldrich), mouse anti-glucagon (Sigma G-2654, 1:100; Sigma-Aldrich), donkey anti-guinea pig Cy3 (706-165-148, 1:500; Jackson ImmunoResearch Laboratories), donkey anti-mouse FITC (715-095-150, 1:200; Jackson ImmunoResearch Laboratories), rabbit caspase-3 antibody (1:50; BD Biosciences), and donkey anti-rabbit FITC secondary antibody (1:100; Jackson ImmunoResearch Laboratories). For ex vivo experiments, stock solution of ranolazine was made in dimethyl sulfoxide and immediately frozen at −80°C before use and then diluted with KRB buffer (129 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO4, 1.2 mM KH2PO4, 2.5 mM CaCl2, 5 mM NaHCO3, 0.1% bovine serum albumin, 10 mM HEPES, pH 7.4) to make various concentrations.

**Data Analysis.** Data are reported as mean ± S.E.M. where designated. Analyses of metabolic parameters were performed using a two-tailed, unpaired Student's t test or analysis of variance followed by Bonferroni’s test. p value <0.05 is considered statistically significant.

## Results

**Ranolazine Lowers FPG and HbA1c Levels in Diabetic Mice.** The time course of the effect of ranolazine on body weight, FPG, and HbA1c is shown in Fig. 1. Ranolazine effectively prevented body weight loss during the week 1 after STZ injection. After this period, mice in both groups showed similar trends in body weight gain through 8-week treatment (STZ + vehicle: 23 ± 0.3 g versus STZ + ranolazine: 24 ± 0.7 g) (Fig. 1A). FPG increased significantly with time in both STZ + vehicle and STZ + ranolazine groups after STZ injection (STZ + vehicle group: baseline 108 ± 3 to 342 ± 29 mg/dl at week 4, STZ + ranolazine group: baseline 115 ± 2 to 264 ± 30 mg/dl at week 4), demonstrating that mice in both groups developed diabetes. However, from weeks 6 to 8, FPG was significantly lower in mice treated with ranolazine than that of the mice in the vehicle group.
(STZ + vehicle: 273 ± 23 mg/dl versus STZ + ranolazine: 188 ± 20 mg/dl, p < 0.05) (Fig. 1B), suggesting that ranolazine slows the progression of diabetes. HbA1c levels also increased significantly in STZ-induced diabetic mice at weeks 4 and 8 compared with baseline; however, HbA1c levels in STZ + ranolazine group were significantly lower than those in STZ + vehicle group after 4- and 8-week treatment (at week 8 STZ + vehicle: 5.8 ± 0.4% versus STZ + ranolazine: 4.6 ± 0.2%, p < 0.05) (Fig. 1C), consistent with the observation in FPG.

Long-Term Treatment of Ranolazine Improves Rapid Insulin Secretion. To investigate the effect of long-term treatment with ranolazine on glucose disposal, an oral glucose tolerance test was performed in STZ + vehicle and STZ + ranolazine groups after 8 weeks of treatment. Overall, glucose levels were lower in ranolazine-treated groups compared with vehicle-treated group (Fig. 2A); however, no difference was observed in plasma concentration–time of glucose curves (i.e., area under the curve for glucose disposal) between the two groups (Fig. 2B).

In fasting state, insulin is secreted constantly at a low basal level. Postprandial in vivo insulin secretion is biphasic with a rapid and transient (within 10 min) burst followed by a second phase that is characterized by sustained flat or gradual decrease that could last 2 to 3 h (Bratanova-Tochkova et al., 2002). Baseline insulin levels were similar in STZ + vehicle (0.25 ± 0.04 ng/ml) and STZ + ranolazine (0.3 ± 0.05 ng/ml) groups. Insulin levels in both groups increased after glucose injection; however, mice treated with ranolazine had significantly (p < 0.05) higher insulin levels (0.54 ± 0.06 ng/ml) compared with that of STZ + vehicle (0.34 ± 0.05 ng/ml) at 15 min (Fig. 2C).

Ranolazine Has No Effect on Insulin Sensitivity in Diabetic Mice. To determine the long-term effect of ranolazine on insulin sensitivity, insulin tolerance test was performed in STZ + vehicle and STZ + ranolazine groups after 8 weeks of treatment. Mice were fasted for 4 h and then given an intraperitoneal injection of insulin (1 U/kg). As shown in Fig. 3A, glucose levels decreased in response to insulin injection in both groups, and there was no difference in glucose disposal curves between the two groups (Fig. 3B), suggesting that ranolazine treatment has no significant effect on insulin sensitivity.

Ranolazine Preserves Islet Morphology and β-Cell Mass in Diabetic Mice. To further explore the mechanism underlying the beneficial effect of ranolazine on FPG and HbA1c, islet morphology was examined using both H&E and immunostaining. Pancreases from age- and sex-matched normal mice were also stained as normal control for comparison with STZ + vehicle and STZ + ranolazine groups. As shown in Fig. 4A, STZ treatment severely decreased β-cell mass and disrupted the islet architecture. The clear round islet boundary was destroyed, and islet shrinkage was observed in STZ + vehicle group compared with healthy islets of the normal group. Treatment with ranolazine partially prevented the shrinkage of the islets. This result was further confirmed by fluorescence staining of insulin-expressing β-cells and glucagon-expressing α-cells (Fig. 4B). The percentages of total islet (insulin and glucagon) area in STZ + vehicle and STZ + ranolazine were 0.21 ± 0.02 and 0.30 ± 0.03%, respectively (p < 0.01) (Fig. 5A). In STZ + vehicle group, insulin-positive area (red staining) was significantly decreased (50 ± 4.6% per islet), whereas glucagon-positive area (green staining) was increased (50 ± 2.1% per islet) compared with the normal group. Ranolazine treatment significantly increased insulin-positive area (69 ± 2.4%, p < 0.05) compared with STZ + vehicle group, suggesting partial preservation of functional β-cell mass in pancreas.

Ranolazine Reduces Apoptosis in Pancreatic Islets in Diabetic Mice. To determine the role of apoptosis in STZ-induced pancreatic morphological changes, we assessed...
pancreatic histological sections for the presence of apoptotic cells. Activated caspase-3, a well recognized apoptosis marker, was measured using immunohistochemistry and presented as percentage of caspase-3 positive area per islet. As shown in Fig. 6, A and B, caspase-3-positive area (24 ± 3.5%) was significantly increased in STZ/vehicle group compared with the normal group (6 ± 0.8%). Ranolazine treatment significantly reduced caspase-3-positive area to 14 ± 1.6% in diabetic mice (p < 0.05 versus STZ/vehicle group).

Ranolazine Increases GSIS. We also investigated whether ranolazine has an effect on GSIS in isolated islets. Rat pancreatic islets were stimulated with 20 mM glucose in the absence or presence of various concentrations of ranolazine for 1 h. As expected, an increase in glucose from 3 to 20 mM caused a 2.5-fold increase in insulin release (Fig. 7). Acute static incubation of rat pancreatic islets with various concentrations of ranolazine (1 nM–1 μM) in the presence of 20 mM glucose caused a further 1.4- to 2-fold increase in GSIS in a concentration-dependent manner, with a maximal effect observed at 1 μM (Fig. 7). Similar effects of ranolazine were observed qualitatively in human pancreatic islets. As shown in Fig. 8A, ranolazine greatly potentiated GSIS in human isolated islets, although the maximal increase in glucose-induced insulin release was observed at 5 μM ranolazine. To determine whether this effect of ranolazine is glucose-dependent, human islets were incubated with various concentrations of glucose in the absence or presence of 1 μM ranolazine. As shown in Fig. 8B, ranolazine had no effect on basal insulin secretion but significantly augmented GSIS under high glucose concentrations. The magnitude of the ranolazine-induced GSIS was approximately 15% higher than that achieved by 50 nM incretin hormone glucagon-like-peptide-1(7–36) amide (Fig. 8C); however, that difference did not reach a statistical significance.

**Discussion**

Ranolazine is a novel anti-ischemic and antianginal drug that increases exercise duration and time to angina episodes in patients with chronic angina pectoris. Timmis et al. (2006) and Morrow and colleagues (Scirica et al., 2007) have shown previously that ranolazine also has beneficial metabolic effects in diabetic subjects as detected by significant reductions of HbA1c in clinical trials. However, the mechanism of this HbA1c-lowering effect remains unknown.

One objective of the present study was to reproduce the antidiabetic effect of ranolazine, observed in humans, in an animal...
model that can then be used to study the mechanism of the antidiabetic effects of ranolazine. By use of a diabetic mouse model induced with multiple low doses of STZ, which causes mild to moderate levels of diabetes as a result of destruction of pancreatic β-cells, we found that 8-week treatment with ranolazine reduces the severity of diabetes as evidenced by lowering of FPG and HbA1c and improving pancreatic β-cell morphology and mass and islet cell survival.

STZ is a widely used diabetogenic agent that produces a selective toxic effect on pancreatic β-cells and induces diabetes mellitus in most laboratory animals (LeDoux et al., 1986). High doses of STZ induce rapid and complete insulin deficiency resembling type 1 diabetes with ketosis. However, lower doses of STZ, which cause a partial destruction of β-cell mass, can be used to produce a mild insulin deficiency resembling type 2 diabetes without a tendency to cause ketosis (Portha et al., 1989). STZ given in multiple low doses can activate immune mechanisms in animals. It induces inflammation of the islets by recruitment of mononucleates and macrophages from extra-islet area with subsequent destruction of islet β-cells within a few days (Papaccio et al., 1991, 1993, 2000). Numerous doses of STZ have been reported to induce diabetes in various non-human primate species, and multiple low doses of 40 mg/kg have been reported to induce β-cell destruction in mice (Takamura et al., 1999; Sun et al., 2005). Results from the present study are consistent with previous findings in the literature (Takamura et al., 1999; Sun et al., 2005). Mice treated with 40 mg/kg STZ for 5 days gradually developed diabetes within 14 days, showing significant increase in FPG levels (Fig. 1B) and HbA1c (Fig. 1C). Histological analysis of the pancreas using either H&E (Fig. 4A) or fluorescent staining (Fig. 4B) showed the collapse of islets. The total number of islets in whole pancreas (Fig. 5A) and percentage of β-cells in each islet (Fig. 5B) significantly declined after treatment with STZ. Islet apoptosis rate was significantly higher in STZ mice compared with healthy controls (Fig. 6). Therefore, we have reproduced a stable diabetic mouse model to investigate the effects of ranolazine in vivo.

In the current study, we report that long-term treatment with ranolazine resulted in significant improvement in glycemic control. Fasting plasma glucose levels represent the most common means of assessing metabolic control in diabetes. Ranolazine-treated STZ mice had significantly lower FPG at 6 weeks compared with the vehicle-treated mice, and

![Fig. 5](image-url) Quantification of total islet area (A), insulin-expressing β-cells (B), and glucagon-expressing α-cells (C) in pancreas from normal mice and STZ-induced diabetic mice treated with vehicle or ranolazine for 8 weeks. Normal group, n = 3, STZ + Vehicle and STZ + ranolazine groups, n = 6. Data are expressed as mean ± S.E.M. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

![Fig. 6](image-url) A, fluorescent staining (20×) of activated caspase-3 in pancreas from normal, STZ + vehicle, and STZ + ranolazine groups. B, apoptosis rate in islets from normal mice and STZ-induced diabetic mice treated with vehicle or ranolazine for 8 weeks. Normal group, n = 3, STZ + Vehicle and STZ + ranolazine groups, n = 6. Data are expressed as mean ± S.E.M. *, p < 0.05; **, p < 0.01.

![Fig. 7](image-url) GSIS in rat pancreatic islets. Freshly isolated rat islets were stimulated with various concentrations of ranolazine (R) in the presence of 20 mM glucose (Glu) in KRB buffer for 60 min at 37°C. Data are expressed as mean ± S.E.M. of 12 experiments in duplicate each. *, p < 0.05 versus 3 mM glucose-treated islets; †, p < 0.05 versus 20 mM glucose-alone-treated islets.
this effect persisted until the end of the experiment (8-week treatment). HbA1c is a standard measure of long-term glycemic control and is used for monitoring the efficacy of the antidiabetic drugs (Derr et al., 2003). Reduction of HbA1c with intensive hypoglycemic therapy is associated with a reduction in complications of diabetes [UK Prospective Diabetes Study (UKPDS) Group, 1998a,b; Stratton et al., 2000]. We found that 8-week treatment with ranolazine at a dose of 20 mg/kg twice a day significantly lowered HbA1c levels (at week 8 STZ + vehicle: 5.8 ± 0.4% versus STZ + ranolazine: 4.6 ± 0.2%, p < 0.05) in STZ-induced diabetic mice (Fig. 1C). This beneficial effect on glycemic control from ranolazine treatment is similar to that seen with conventional dipeptidyl peptidase-4 inhibitor sitagliptin (Mu et al., 2006), which treatment is similar to that seen with conventional dipeptidyl peptidase-4 inhibitor sitagliptin (Mu et al., 2006), which reduced HbA1c from 7.5 ± 0.2 to 5.6 ± 0.3% after 2 to 3 months of treatment in STZ-induced diabetic mice on high-fat diet.

The deterioration of glycemic control over time in type 2 diabetes is believed to be linked to the progressive loss of β-cell function and mass (Wajchenberg 2007). The major finding of this study is that long-term treatment of ranolazine slows the progression of diabetes induced by STZ by increasing GSIS and preserving β-cell mass in diabetic mice. Ranolazine treatment increased peak insulin secretion at 15 min during an OGTT at 8 weeks (Fig. 2B), indicating some improvement of first phase of insulin secretion from existing β-cells. Ranolazine treatment preserved β-cell mass and decreased apoptosis rate (STZ + Vehicle: 23 ± 3.6% versus STZ + ranolazine: 14 ± 1.5%) in the islets (Figs. 4, 5, and 6), suggesting that long-term treatment with ranolazine in STZ-induced diabetic mice ameliorates the progression of diabetes by targeting pancreatic β-cells. Thus, the protective effect of ranolazine in β-cell preservation may be the mechanism to maintain normal glycemia after STZ treatment. Similar findings have been reported with exendin-4 and rosiglitazone. Exendin-4 significantly reduced (4.5-fold) the numbers of apoptotic β-cells in mice administered both STZ and exendin-4 compared with that in STZ-treated mice (Li et al., 2003). Rosiglitazone also improved glucose homeostasis by significantly decreasing apoptosis rate (percentage) of β-cells in rosiglitazone-treated STZ-induced diabetic rat compared with that of nontreated STZ group (6.52 ± 0.77 versus 10.33 ± 1.07%) (Liu and An, 2009). Other studies have shown that STZ may increase production of inflammatory cytokine interleukin-1β and nitric oxide (Corbett et al., 1992a,b; Kwon et al., 1994), which may contribute to STZ-induced β-cell apoptosis and diabetes. Exendin-4 has been reported to inhibit interleukin-1β and nitric oxide production in islet cells via the cAMP/protein kinase A system (Kang et al., 2009). Whether ranolazine has any beneficial effect in regulation of inflammatory cytokines and oxidative stress in vivo will be investigated in future studies.

Consistent with in vivo findings that ranolazine treatment increased transient insulin secretion during OGTT in diabetic mice, data from our ex vivo studies demonstrate that ranolazine directly induces rapid glucose-dependent insulin secretion from rat and human isolated islets as reported previously (Dhalla et al., 2008). Therefore, in addition to β-cell preservation, the effects of ranolazine on FPG and HbA1c in diabetic mice could also be due in part to improved insulin secretion from the existing residual β-cells. It has been well characterized that glucose induces insulin secretion through glycolysis and mitochondrial oxidation in the cells, which increase intracellular ATP/ADP ratio, sequentially leading to closure of KATP channels, depolarization of voltage-gated L-type Ca2+ channels on the plasma membrane, Ca2+ influx, and activation of exocytosis of insulin-containing granules (Rutter 2001; Iezzi et al., 2004; Newsholme et al., 2005). Although the exact mechanism of increased insulin secretion by ranolazine is not known, ranolazine significantly stimulated insulin secretion only in the presence of high glucose but had minimal or no effect at low glucose (Fig. 8B), suggesting that ranolazine does not increase insulin secretion via KATP channel-like sulfonlurea. Therefore, the risk of hypoglycemia (a major concern with sulfonlurea therapy) with ranolazine treatment may be very low. Ranolazine inhibits INa and IKr in cardiace cells; the two ion currents have also been shown to play a role in insulin secretion in β-cells. Inhibition of INa by ranolazine may increase the concentration of intracellular ATP by lowering the consumption of ATP (Ding and Kitsato 1997), thereby inhibiting IKATP and causing depolarization of the β-cell followed by activation of calcium channels, increase in intracellular calcium concentration, and consequent increase in insulin release. Inhibition of IKr by ranolazine may decrease outward K+ current, and this sustains the repolarization of...
the β-cells that can result in activating calcium channels and increasing in intracellular calcium concentration followed by an increase in insulin release (Rosati et al., 2000). Whether these properties of ranolazine play a role in its effect on glucose homeostasis remains to be determined. In addition to the β-cell preservation and increased insulin secretion in the pancreas, ranolazine may also have effect on other organs, such as liver and skeletal muscle, which are currently under investigation.

A limitation of our study is that we did not have a positive control group; therefore, we can not directly compare the magnitude of the effect of ranolazine in this study with other drugs. Furthermore, the effects of ranolazine may be underestimated in this study because the half-life of ranolazine in rodents is approximately 2 h; thus, dosing the mice twice daily results in therapeutic levels of the drug (5–10 μM) for a few hours only (plasma concentrations of ranolazine at 15, 30, and 60 min after oral dosing were: 9.1 ± 3.3, 7.4 ± 3.0, and 6.1 ± 2.8 μM, respectively). To address this issue, a follow-up study, in which ranolazine concentration will be maintained at therapeutic levels in animals by giving the drug in diet, is planned.

In summary, the data from the present study replicate the antiangiogenic effects of ranolazine seen in clinical trials. We provide evidence for the first time that ranolazine may exert an antiangiogenic effect by protecting pancreatic β-cell mass and improve insulin secretory function. Loss of functional β-cell mass is the key for deterioration of glycemic control in both type 1 and type 2 diabetes. In this context, ranolazine has the potential to become a novel agent that is capable of treating patients with both angina and diabetes. However, more preclinical studies are needed to further characterize the potential antiangiogenic effect of ranolazine and to unravel the molecular mechanisms underlying this effect.

Authorship Contributions

Participated in research design: Ning, Liu, Dhalla, and Belardinelli.
Conducted experiments: Ning, Zhen, Fu, Jiang, and Liu.
Contributed new reagents or analytic tools: Ning and Jiang.
Performed data analysis: Ning, Fu, and Jiang.
Wrote or contributed to the writing of the manuscript: Ning, Liu, Dhalla, and Belardinelli.

References

Bakris GL, Gaxiola E, Messerli FH, Mancia G, Erdine S, Cooper-DeHoff RM, and Belardinelli.
Conduct experiments: Ning, Zhen, Fu, Jiang, and Liu.
Contributed new reagents or analytic tools: Ning and Jiang.
Performed data analysis: Ning, Fu, and Jiang.
Wrote or contributed to the writing of the manuscript: Ning, Liu, Dhalla, and Belardinelli.


**Address correspondence to:** Dr. Arvinder K. Dhalla, Department of Biology, Gilead Palo Alto Inc., 1651 Page Mill Rd, Palo Alto, CA 94304. E-mail: arvinder.dhalla@gilead.com