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Anti-Adrenergic and Hemodynamic Effects of Ranolazine in Conscious Dogs Zhao, Anti-Adrenergic Effect of Ranolazine

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Abstract

Effects of ranolazine alone and in the presence of phenylephrine (PE) or isoproterenol (ISO) on hemodynamics, coronary blood flow (CBF) and heart rate (HR) in the absence and presence of hexamethonium (a ganglionic blocker) were studied in conscious dogs. Ranolazine (0.4, 1.2, 3.6 and 6 mg/kg, IV) alone caused transient (<1 min) and reversible hemodynamic changes. PE (0.3 to 10 µg/kg) caused a dose-dependent increase in BP and decrease in HR. ISO (0.01 to 0.3 µg/kg) caused a dose-dependent decrease in BP and increase in HR. Ranolazine at moderate (4–5 µM) and high (11–13 µM) concentrations did not affect the changes in MAP and HR caused by either PE or ISO, or partially attenuated these effects, respectively. However, in dogs treated with hexamethonium (20 mg/kg) to cause autonomic blockade, ranolazine (both 4–5 and 11–13 µM) significantly attenuated both the PE- and ISO-induced changes in MAP. The results suggest that a potential anti-adrenergic effect of ranolazine was masked by autonomic control mechanisms in conscious dogs, but could be observed when these mechanisms were inhibited (e.g., in the hexamethonium-treated dog). Ranolazine, at plasma concentrations below 10 µM and in conscious dogs with intact autonomic regulation, had minimal anti-adrenergic (α and β) effects.

Keywords

ranolazine; hemodynamics; anti-adrenergic effect; coronary blood flow; conscious dogs

INTRODUCTION

Ranolazine is used for treatment of patients with chronic stable angina. Results from clinical trials show that ranolazine reduces the frequency of anginal attacks and increases exercise

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tolerance in these patients.¹⁻⁴ In addition, ranolazine is also reported to suppress atrial and ventricular arrhythmias in patients with non-ST-segment-elevation acute coronary syndrome.^{5,6}

The late sodium current (late I_{Na}) in the heart contributes to Na^+ entry in myocytes, and an enhancement of late I_{Na} has been shown to lead to both Na^+ and Ca^{++} loading (Ca^{++} loading occurs as a result of Na^+/Ca^{++} exchange).⁷ It has been well established that ranolazine inhibits the late I_{Na} in cardiac myocytes. Inhibition of late I_{Na} by ranolazine reduces sodium entry into myocytes during the action potential plateau, thereby increasing repolarization reserve and potentially reducing calcium influx via the Na^+/Ca^{++} exchange.⁸⁻¹¹

Ranolazine, in addition to inhibiting late I_{Na} , has been reported to block α_1 -adrenergic receptors with a pKi value of 5.1 in vitro.¹² Furthermore, ranolazine was reported to partially block the effect of isoproterenol (ISO) to increase developed tension in rat isolated atria, and to attenuate ISO-induced increases in heart rate (β_1 receptor) and decreases in blood pressure (β_2 receptor) in pithed rats.¹³ These results suggest that ranolazine has weak effects to antagonize α_1 -, β_1 - and β_2 -adrenergic receptors.

The functional significance of the above mentioned sympatholytic effects of ranolazine in intact and awake animals has not been previously studied. In clinical trials, ranolazine has not been found to reduce either heart rate (HR) or blood pressure (BP) in patients with chronic angina.¹⁻⁴ Therefore, a functional cardiovascular “signal” of potential block by ranolazine of adrenergic receptors has not been demonstrated. Thus, in the present study we investigated the effects of ranolazine at different doses on HR, BP, coronary blood flow and other cardiovascular parameters in conscious dogs. We used the α -adrenergic agonist phenylephrine (PE) and the β -adrenergic agonist isoproterenol (ISO) as pharmacological tools to evaluate the anti-adrenergic properties of ranolazine. However, both PE and ISO cause significant changes in BP that result in a reflex regulation of the cardiovascular system in response to the change in BP, and this reflex response may potentially mask the effect of ranolazine. Therefore, dogs were pretreated with hexamethonium (an autonomic ganglionic blocking agent) in selected experiments to block reflex regulation of the cardiovascular system so that the direct anti-adrenergic effects of ranolazine could be determined in the absence of autonomic nervous control of cardiovascular function.

MATERIALS AND METHODS

Experimental protocols were approved by the Institutional Animal Care and Use Committees of Gilead Sciences, New York Medical College or the University of Florida College of Medicine, and conformed to the Guide for the Use and Care of Laboratory Animals established by the National Institutes of Health.

In Vitro Studies

Radioligand Binding Assay for Adrenergic Receptors—Rat brain, liver, salivary glands or ventricle were dissected and rinsed several times in ice-cold buffer solution containing 50 mM Tris-HCl, 5 mM $MgCl_2$ and 250 mM sucrose (pH 7.5). Tissues were diced and homogenized for 1 min and centrifuged at 40,000g at 4 °C for 10 min. Membrane

pellets were washed, resuspended and centrifuged again. The final membrane pellet was resuspended in the same buffer and used for binding assays. For measurement of ranolazine binding to α -adrenergic receptors, 0.25 nM ^3H -prazosin (Perkin Elmer, Boston, MA) was incubated for 90 min at room temperature with various concentrations of ranolazine and 100 μg membrane protein in TE buffer (50 mM Tris and 1 mM EDTA). Incubation was terminated by filtration using a Packard harvester and membranes were washed with ice-cold TE buffer four times. Total bound radioligand was determined by scintillation counting. Non-specific binding was determined in the presence of 10 μM phentolamine. For measurement of ranolazine binding to rat ventricular β_1 -adrenergic receptors, 0.5 nM ^3H -CGP-12177 (Perkin Elmer, Boston, MA) was incubated for 20 min at room temperature with various concentrations of ranolazine and 200 μg rat ventricle membrane homogenate in buffer containing 20 mM Tris-HCl, 154 mM NaCl, 2 mM MgCl_2 , and 0.1 mM GTP in the presence of 10 nM ICI 118,551 (to block the β_2 -receptor binding sites). Non-specific binding was determined in the presence of 10 μM propranolol. For measurement of ranolazine binding to lung β_2 -adrenergic receptors, 0.5 nM ^3H -CGP-12177 (Perkin Elmer, Boston, MA) was incubated for 20 min at room temperature with various concentrations of ranolazine and 100 μg membrane homogenate of guinea pig lung in buffer containing 20 mM Tris-HCl, 154 mM NaCl, 2 mM MgCl_2 , and 0.1 mM GTP in the presence of 10 nM CGP 20712A (to block the β_1 -receptor binding sites). Non-specific binding was determined in the presence of 10 μM propranolol.

In Vivo Studies

Surgical Procedures—Dogs were sedated with acepromazine (0.3 mg/kg, IM) and anesthetized with pentobarbital sodium (25 mg/kg, IV), intubated, and artificially ventilated with room air. The chest of each dog was scrubbed with a sterilizing soap and sterilized with iodine solution. A thoracotomy was performed in the fifth intercostal space. A Tygon catheter (Cardiovascular Instruments, Wakefield, MA) was inserted into the descending thoracic aorta for measurement of mean arterial blood pressure (MAP) and for blood sampling. A solid-state pressure gauge (P6.5, Konisberg Instrument, Inc, Pasadena, CA) was implanted into the left ventricle through the apex for measurement of left ventricular pressure (LVP). In some dogs, a Doppler flow transducer (Craig Hartley, Houston, TX) was placed around the left circumflex coronary artery for measurement of the velocity of coronary blood flow (CBF). The chest was closed in layers. The catheter and wires were tunneled subcutaneously and externalized through the skin at the back of the dog's neck. After surgery, no procedures were done for 10-14 days to allow the dogs to recover from the surgery. Dogs were trained to lie quietly on a laboratory table. On the experimental day, catheters were inserted into a peripheral vein in one front and one rear leg to administer drugs. Dogs lay undisturbed, either awake or asleep, during the experiment.

Coronary Blood Flow and Hemodynamic Measurements—Phasic arterial pressure was measured by connecting the previously implanted aortic catheter to a strain gauge transducer (P23 ID, Statham, Newark, NJ), and MAP was derived using a 2 Hz low-pass filter. HR was determined from pressure pulse intervals using a cardiometer (Beckman Coulter, Inc, Fullerton, CA). Left ventricular pressures were measured by the solid-state pressure gauge and $\text{LV } +\text{dP/dt}$ was calculated by a differentiator having a frequency

response flat to 700Hz. The velocity (cm/s) of coronary blood flow was measured using a pulsed Doppler flowmeter (System 6, Triton, San Diego, CA). The flow volume (mL/min) was calculated per vendor's instruction. Mean coronary vascular resistance (MCVR) = MAP/CBF. In some dogs, the standard limb lead II of the electrocardiogram (ECG) was recorded. All parameters were recorded on a paper recorder at speeds of 25 or 50 mm/sec as needed.

Effects of Ranolazine Alone on Hemodynamics and Coronary Circulation

Four doses of ranolazine were administered intravenously, using a 30-sec bolus iv injection followed by a 1 mL/min iv infusion, to achieve target blood concentrations of 1, 3, 9 or 15 μ M.

The dosing regimens were:

Dose 1: 0.4 mg/kg bolus iv injection followed by 22.5 μ g/kg/min infusion for 15 min.

Dose 2: 1.2 mg/kg bolus iv injection followed by 67.5 μ g/kg/min infusion for 15 min.

Dose 3: 3.6 mg/kg bolus iv injection followed by 200 μ g/kg/min infusion for 15 min.

Dose 4: 6.0 mg/kg bolus iv injection followed by 337.5 μ g/kg/min infusion for 15 min.

The steady-state plasma ranolazine concentrations measured in blood samples from dogs that received those doses of ranolazine were 1.1, 3.4, 11-13 and 17-18 μ M, respectively (Tables 1 and 2). Those concentrations were slightly higher than the targeted concentrations. Recordings of the measured parameters (e.g., HR, BP, CBF, etc.) were obtained at 0, 5, 10 and 15 min following each ranolazine infusion, and blood samples were withdrawn at each time. Ranolazine concentrations in the plasma were measured using standard LC/MS-MS.

Anti-Adrenergic Effects of Ranolazine

To determine the effects of ranolazine on BP and HR in conscious dogs treated with either PE or ISO, the following protocols were used. First, the responses of dogs to bolus IV injections of PE at ascending doses of 0.3, 1.0, 3.0 and 10 μ g/kg, or ISO at ascending doses of 0.01, 0.03, 0.1 and 0.3 μ g/kg were recorded. Then ranolazine was administered to achieve a steady-state plasma concentration of either 4-5 or 11-13 μ M. This was followed by a second series of iv injections of either PE or ISO in the continued presence of ranolazine. To achieve a steady-state ranolazine plasma concentration of 4-5 μ M, an iv bolus of 1.6 mg/kg and a 30-40 min infusion at a rate of 90 μ g/kg/min were used. To achieve a steady-state ranolazine plasma concentration of 11-13 μ M, an iv bolus of 3.6 mg/kg bolus followed by a 30-40 min infusion at a rate of 200 μ g/kg/min were used. To measure plasma ranolazine concentrations, blood samples were taken from the aortic catheter at 10 min after the initiation and prior to stopping the ranolazine infusion (i.e., 30-40 min after the initiation of the infusion).

To study the effects of ranolazine on BP and HR in conscious dogs after blockade of autonomic nervous transmission, hexamethonium (20 mg/kg, iv) was administered, followed 30-40 min later by either PE or ISO alone in a series of ascending doses, followed by

ranolazine (4-5 or 11-13 μM), followed by PE or ISO again in the continued presence of ranolazine.

Data Collection and Statistical Analysis

Values of MAP, HR, LVSP, and CBF were obtained from the strip-chart records before (baseline) and after ranolazine administration, at peak response and at 5, 10 and 15 min after the initiation of the infusion. The rate of rise of LV systolic pressure ($\text{LV } +\text{dP}/\text{dt}$) was calculated from the strip-chart record of LVSP. Mean coronary vascular resistance (MCVR) was calculated as the ratio of MAP to CBF. R-R and QT intervals of the ECG were measured from the strip-chart record with a ruler. The statistical significance of a difference between the value of a parameter at baseline and the value of a parameter at peak response after drug administration was determined using a paired t-test. The statistical significance of a difference between the value of a parameter at baseline and the value at an indicated time point after ranolazine iv infusion was determined using a one-way repeated ANOVA. Statistical significance of differences between responses to PE or ISO in the absence and presence of ranolazine was determined using a two-way ANOVA followed by Tukey's test. Differences associated with a probability (p) < 0.05 were considered to be significant. A computer-based software package (SigmaStat 2.03, SPSS Science, Chicago, IL) was used for statistical analysis. All data are presented as mean $\pm$ SEM.

RESULTS

Affinities of Ranolazine for α - and β -Adrenergic Receptors

Ranolazine bound to both α - and β -adrenergic receptors with micromolar affinity (Table 1). As summarized in the table, the K_i values for ranolazine to bind to α - and β -adrenergic receptors ranged from a low of $8.2 \pm 1.8 \mu\text{M}$ for binding to α_{1A} receptors in membranes from rat salivary gland to a high of $19.5 \pm 1.4 \mu\text{M}$ for binding to α_{1B} in membranes from rat liver.

Effects of Ranolazine Alone on Hemodynamics and Coronary Circulation

Bolus (over 30 sec) iv injections of ranolazine caused transient (30-45 sec) and dose-dependent increases in CBF, HR and $\text{LV } +\text{dP}/\text{dt}$, and dose-dependent decreases in MCVR, LVSP, and MAP. Figure 1 shows an actual recording from one dog and Figure 2 shows summarized data from 5-6 dogs. In contrast to the acute and transient effects, the steady-state effects of ranolazine at 4 different doses were minimal (Tables 2 and 3). Ranolazine at steady-state concentrations from 1 to 18 μM did not significantly affect LVSP, MAP, HR or CBF compare to baseline (Table 2 and 3).

In the absence of hexamethonium, a bolus iv injection of 3.6 mg/kg ranolazine caused a transient peak decrease in MAP by $20 \pm 3 \text{ mmHg}$ and a transient peak increase in HR by $82 \pm 15 \text{ bpm}$ from baseline (both $p < 0.05$). In the presence of hexamethonium, the same dose of ranolazine (3.6 mg/kg) decreased MAP by $42 \pm 3 \text{ mmHg}$ ($p < 0.05$), but did not cause a significant change in HR (HR: $2 \pm 2 \text{ bpm}$, $p > 0.05$, compared to baseline).

At 5, 10 and 15 min following the administration of ranolazine at doses 1, 2 and 3 (steady-state plasma ranolazine concentrations of 1.1, 3.4-3.5 and 10.6-13.6 μM , respectively), the values of LSVP, LV +dP/dt, MAP, HR, CBF and MCVR were not significantly different from baseline values of the same parameters. The highest dose (dose 4, plasma ranolazine concentrations of 16.7-17.7 μM) of ranolazine caused a 15% decrease in LV +dP/dt at 5 min ($p < 0.05$, compared to baseline), but changes in LVSP, MAP, HR, CBF and MCVR were not significant (all $p > 0.05$, compared to baseline). Ranolazine administration at any tested dose did not cause significant changes from baseline in the R-R and QT intervals of the ECG (Table 3). During the infusion of ranolazine, there were no observable changes in the dogs' behavior. The animals lay quietly on the laboratory table without any signs of discomfort or distress.

Anti-Adrenergic Effects of Ranolazine

The anti-adrenergic effects of two doses ("low" and "high") of ranolazine (Table 4) were studied. The low and high doses engendered plasma ranolazine concentrations of 4 ± 0.2 to 5 ± 0.3 and 11 ± 2 to 14 ± 0.8 μM , respectively, as summarized in Table 4.

Effects of Ranolazine on Changes in Hemodynamics Induced by PE or ISO in Intact Conscious Dogs—Bolus iv injections of PE at doses of 0.3, 1.0, 3.0 and 10 $\mu\text{g/kg}$ caused a dose-dependent increase in MAP and a dose-dependent decrease in HR (Figure 3, all $p < 0.05$). The PE-induced increases in MAP and decreases in HR were not significantly altered by 4–5 μM ranolazine. Thus, as shown in Figure 3, in the presence of the lower concentration of ranolazine (4–5 μM), PE at doses of 0.3, 1.0, 3.0 and 10 $\mu\text{g/kg}$ still increased MAP and decreased HR. However, at the higher concentration (11–13 μM), ranolazine partially and significantly attenuated the PE-induced increases in MAP ($p < 0.05$, compared with control). Interestingly, the PE-induced decreases in HR were not markedly altered by 11–13 μM ranolazine (Figure 3), suggesting that did not alter the baroreflex regulation of HR caused by an increase in MAP. Bolus iv injections of ISO at doses of 0.01, 0.03, 0.1 and 0.3 $\mu\text{g/kg}$ caused dose-dependent decreases in MAP and dose-dependent increases in HR (Figure 4, all $p < 0.05$). The ISO-induced changes in MAP and HR were not significantly altered by 4–5 μM ranolazine (Figure 4), although the increases in HR by ISO were slightly less in the presence than in the absence of ranolazine. As shown in Figure 4, at the higher concentration (11–13 μM), ranolazine partially and significantly attenuated the ISO-induced changes in MAP and HR ($p < 0.05$, compared with control).

Effects of Ranolazine on Changes in MAP and HR Induced by PE or ISO in Conscious Dogs after Treatment with Hexamethonium—There were a significant decrease in MAP (105 ± 2 vs 85 ± 2 mmHg, $p < 0.05$) and a significant increase in HR (95 ± 3 vs. 135 ± 3 mmHg, $p < 0.05$) following administration of hexamethonium to block transmission in autonomic ganglia in the dog. After treatment of dogs with 20 mg/kg hexamethonium, the MAP response to a given dose of PE was enhanced compared to control (compare control values in Figures 3 and 5), whereas the HR response to PE was much reduced (no significant change compared to baseline; data not shown). Ranolazine (4–5 μM and 11–13 μM) significantly attenuated the PE-induced increases in MAP in the presence of hexamethonium (Figure 5). After treatment of dogs with hexamethonium (20 mg/kg), the

ISO-induced decrease of MAP was enhanced by approximately 10-fold compared to control (compare control values in Figures 4 and 6). Bolus iv injections of ISO at doses of 0.001, 0.003, 0.01 and 0.03 µg/kg caused a dose-dependent decrease in MAP and a dose-dependent increase in HR (Figure 6) in the presence of hexamethonium. The ISO-induced decrease in MAP and increase in HR were significantly attenuated by 4–5 µM and 11–13 µM ranolazine (Figure 6).

DISCUSSION

Ranolazine at steady-state plasma concentrations of 1 to 18 µM did not significantly alter LVSP, MAP, HR or CBF in conscious dogs. However, we found that ranolazine has weak anti-adrenergic effects that can be unmasked when autonomic reflex regulation of cardiovascular function is absent. Within the therapeutic concentration range of 2 to 6 µM, ranolazine alone did not exert a significant anti-adrenergic effect in the conscious dog. However, after blockade of autonomic transmission with hexamethonium, a significant anti-adrenergic effect of ranolazine (4–5 µM) could be demonstrated.

Immediately following a bolus iv injection of ranolazine, brief (< 1 min) increases in HR, LV +dP/dt and CBF, and decreases in MAP and LVSP were observed (Figure 1). Following treatment of the dog with hexamethonium, the acute effect of ranolazine to decrease MAP was exaggerated, whereas the effect of ranolazine to increase HR was absent. These findings suggest that the direct effect of acute iv ranolazine administration is to cause vasodilation, whereas the ranolazine-induced increase of HR is secondary to activation of an autonomic baroreflex that is blocked by hexamethonium. The present study showed that iv injection of ranolazine caused a transient (<1 min) and dose-dependent increase in CBF, which is consistent with the results from a recent report.¹⁴

As expected, bolus iv injections of PE (0.3 to 10 µg/kg) caused a dose-dependent increase in MAP by up to 66 mmHg and a dose-dependent decrease in HR by up to 41 bpm. On the other hand, bolus iv injections of ISO (0.01 to 0.3 µg/kg) dose-dependently decreased MAP by up to 43 mmHg and increased HR by up to 160 bpm, consistent with the findings of previous studies of conscious dogs.^{15–18} The results of the present study indicated that 4–5 µM ranolazine did not affect the PE/ISO-induced changes in BP and HR. However, 11–13 µM ranolazine did partially and significantly attenuate the PE- or ISO-induced changes in MAP and HR in the intact conscious dog, and this sympatholytic effect of ranolazine was enhanced in dogs treated with hexamethonium. Data from clinical trials showed that the highest recommended therapeutic dose of ranolazine (1000 mg bid) yielded a peak concentration of 2 to 6 µM.^{1, 2, 7} Thus, the anti-adrenergic effects of ranolazine are likely to be observed when the plasma concentration of ranolazine is higher than that usually achieved therapeutically, or when autonomic reflexes are markedly suppressed such as during anesthesia or when autonomic regulation is impaired.

Both PE and ISO can cause significant changes in BP in dogs. In dogs with an intact autonomic nervous system, the change in BP causes a change in HR mediated by the arterial baroreflex to bring BP back to its pre-existent level. This reflex action is mediated by sympathetic and parasympathetic (vagal nerves). The reflex change in HR, in turn, partially

antagonizes the direct change in BP induced by PE or ISO.^{17,18} In the present study, hexamethonium was used to block the reflex effects induced by PE or ISO. The results showed that hexamethonium effectively or near completely blocked transmission in autonomic ganglia, as indicated by the finding that after hexamethonium administration, PE did not cause a significant change in HR, although it still caused a dose-dependent increase in MAP. The MAP responses to PE and ISO were enhanced in conscious dogs treated with hexamethonium, suggesting that the change in HR mediated by the arterial baroreflex partially attenuates the direct actions of PE/ISO on MAP. After treatment of dogs with hexamethonium, both 4-5 and 11-13 μM ranolazine partially attenuated the changes in MAP elicited by PE. Furthermore, after treatment of the dog with hexamethonium, ranolazine at both 4-5 and 11-13 μM significantly reduced the ISO-induced changes in MAP and HR as well. The present results are consistent with the results from the binding data (Table 1) and a previous study, in which it was reported that ranolazine significantly attenuated the decrease in diastolic arterial pressure and the increase in HR induced by ISO in a dose-dependent manner (2.5-80 mg/kg) in pithed rats.¹³ The present results suggest that 4-5 μM ranolazine has a weak effect to antagonize α - and β -adrenergic receptors. In the intact conscious dog, this weak antagonism of α - and β -adrenergic receptors is masked by autonomic nervous activity that serves to regulate BP. However, after the near complete blockade of transmission in autonomic ganglia with hexamethonium, the hemodynamic effect of ranolazine's antagonism of α - and β -adrenergic receptors is unmasked. This may be explained by the fact that ranolazine is a weak competitive antagonist of adrenergic receptors. Previously published results indicate that ranolazine has micromolar affinity for α - ($\text{pK}_i=5.1$)¹² and for β_1 - and β_2 -adrenergic receptors ($\text{pK}_i=5.8$ and 6.3, respectively) in rat hearts and guinea-pig lungs.¹² Using similar assays, we also found that ranolazine bound to α - and β -adrenergic receptors in different rat tissues-- K_i values for ranolazine to antagonize α - adrenergic receptors were 8.2 to 19.5 μM (Table 1), and to antagonize β_1 - and β_2 -adrenergic receptors were 8.6 and 14.8 μM , respectively (Table 1). Using the K_i values and plasma concentrations from the present study, in the absence of any competitor, α -adrenergic receptor occupancies by ranolazine would be about 25 and 50% at concentrations of 4-5 μM and 11-13 μM , respectively. Thus, only the higher concentration of ranolazine is likely to exert a significant anti-adrenergic effect, by competing with the endogenous adrenergic receptor agonists. Indeed, there are "tonic" activities from the central nervous system to peripheral vessels to cause vasoconstriction in physiological conditions,¹⁸ as indicated in the present study by the finding that blood pressure was significantly reduced after administration of hexamethonium to block autonomic nervous transmission. Taken together, the results of in vitro and in vivo experiments suggest that sympathetic tone and/or occupancy by endogenous agonists of adrenergic receptors is sufficient to prevent the anti-adrenergic effect of 4-5 μM ranolazine, but that higher concentrations of ranolazine, or reduction of autonomic nervous activity, may unmask the anti-adrenergic effect of the drug. An alternative mechanism for the anti-adrenergic effects of ranolazine may be related to the reduction of α_1 -adrenergic receptors numbers by ranolazine from an earlier report.¹⁹

Limitation of the Study

In this study, we found that bolus iv injections of ranolazine resulted in transient (<1 min) and dose-dependent decreases in MAP and LVSP and increases in CBF, HR and LV +dP/dt.

The mechanism(s) underlying these transient effects following bolus iv injection of ranolazine to alter hemodynamics has not been determined, but it does not seem to contribute to the steady-state effects of the drug.

CONCLUSION

In the intact conscious dog, steady-state plasma concentrations of ranolazine up to 18 μM were not associated with significant changes in hemodynamics, coronary circulation or R-R and QT intervals. However, a bolus iv injection of ranolazine caused transient ($< 1\text{min}$), reversible and dose-dependent decreases in LVSP, MAP and MCVR, and increases in LV +dP/dt, HR and CBF. Changes in BP and HR induced by stimulation of α - and β -adrenergic receptors were not significantly altered by 4–5 μM ranolazine, whereas they were significantly attenuated by 11–13 μM ranolazine. After blockade of transmission in autonomic ganglia with hexamethonium, ranolazine at both 4–5 and 11–13 μM concentrations significantly attenuated changes in MAP and HR induced by stimulation of α - and β -adrenergic receptors. In the hexamethonium-treated dog, ranolazine reduced MAP but did not alter HR, suggesting that the drug caused vasodilation but not a direct effect on HR. The results indicate that ranolazine has weak anti-adrenergic effects that become more evident when autonomic reflex regulation of cardiovascular function is absent.

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Figure 1.

Representative recording shows cardiovascular response to iv injection (over 30 sec) of ranolazine in a conscious dog. LVP, left ventricular pressure, BP, blood pressure, HR, heart rate, CBF, coronary blood flow.

Figure 2.

Bolus iv injections of ranolazine caused a dose-dependent increase in coronary blood flow (CBF, the left top panel) and decrease in mean coronary vascular resistance (MCVR, the left bottom panel), decreases in left ventricular systolic pressure (LVSP, middle top panel), mean arterial pressure (MAP, middle bottom panel) and increases in LV +dP/dt (the right top panel) and heart rate (HR, the right bottom panel). These responses were transient and lasted only 30 to 45 sec. * $p < 0.05$, compared to baseline.

Figure 3.

Bolus iv injections of phenylephrine (PE) caused a dose-dependent increase in mean arterial pressure (MAP, top panel) and decrease in heart rate (HR, bottom panel). In the presence of a steady-state concentration of 4-5 μM ranolazine, the PE-induced increase in MAP was not significantly altered, whereas in the presence of 11-13 μM ranolazine, the PE-induced increase in MAP was partially and significantly attenuated. # $p < 0.05$, control vs. ranolazine.

Figure 4.

Bolus iv injections of isoproterenol (ISO) caused a dose-dependent decrease in mean arterial pressure (MAP) and increase in heart rate (HR). In the presence of 4-5 μM ranolazine, the ISO-induced decrease in MAP and increase in HR were not significantly altered, whereas in the presence of 11-13 μM ranolazine, the ISO-induced decrease in MAP and increase in HR were partially and significantly attenuated. # $p < 0.05$, control vs. ranolazine

Figure 5. Ranolazine significantly attenuated MAP and HR responses to PE in the hexamethonium-treated dog. Hexamethonium (20 mg/kg) was administered by iv injection to block autonomic nervous transmission. # $p < 0.05$, control vs. ranolazine.

Figure 6.

Ranolazine significantly attenuated MAP and HR responses to isoproterenol (ISO) in the hexamethonium-treated dogs. Hexamethonium (20 mg/kg) was administered by iv injection to block autonomic nervous transmission. # $p < 0.05$, control vs. ranolazine.

Table 1

Ki Values of Ranolazine for binding to Adrenergic Receptors

Receptor	n	Tissues	Ki (μM)
α ₁	10	Rat Ventricle	10.5±3.4
α _{1A}	3	Rat Salivary Gland	8.2±1.8
α _{1B}	3	Rat Liver	19.5±1.4
β ₁	3	Rat Ventricle	8.6±2.1
β ₂	6	Guinea Pig Lung	14.8±2.6

Ki = the concentration of competing ligand in a competition assay which would occupy 50% of the receptors if no ligand were present

Table 2

Steady-State Effects of Ranolazine on Hemodynamic Parameters in Conscious Dogs

Parameters [Ran] μ M ¹	n	Baseline	5 min	10 min	15 min	(%)
LVSP (mmHg)						
1.1	5	136±6	125±6	130±7	128±6	-1.5±1.1
3.4	5	126±6	123±8	124±7	124±8	2.0±1.7
11-13	5	126±6	122±6	119±7	123±7	-2.0±1.5
17-18	5	130±6	121±6	126±7	122±4	-6.0±2.2
LV +dP/dt (mmHg/s)						
1.1	5	2669±360	2528±337	2606±338	2571±332	-3.4±1.1
3.4	5	2538±311	2403±312	2415±295	2446±277	-3.2±2.0
11-13	5	2304±200	2218±189	2198±217	2213±209	-4.3±1.7
17-18	5	2570±148	2175±71*	2273±89	2231±129	-13.1±2.1
MAP (mmHg)						
1.1	6	103±5	100±4	100±4	101±4	-2.1±0.7
3.4	6	104±5	100±5	102±5	103±6	-0.4±1.1
11-13	6	103±5	101±5	99±4	100±5	-2.8±1.4
17-18	6	104±3	103±4	103±3	101±3	-3.1±1.7
HR (beats/min)						
1.1	6	98±8	99±8	99±6	95±7	-2.2±5.1
3.4	6	95±7	96±7	99±7	99±8	4.0±4.0
11-13	6	92±7	103±7	96±7	97±7	6.4±5.7
17-18	6	87±8	100±8	104±9	102±9	18.3±6.3

LVSP, Left ventricular systolic pressure; MAP, Mean arterial pressure; HR, Heart rate. Data are Mean ± SEM

¹Steady-state ranolazine plasma concentrations measured at 5-15 min after bolus and infusion of each of 4 doses (for doses, see METHOD).

Table 3
Steady-State Effects of Ranolazine on Coronary Circulation and R-R and QT Intervals in Conscious Dogs

Parameters [Ran] μM ¹	n	Baseline	5 min	10 min	15 min	(%)
CBF (mL/min)						
1.1	5	36±5	37±5	37±5	36±5	0.5±2.2
3.4	5	38±5	38±6	37±5	38±4	-1.4±2.0
11-13	5	37±6	38±5	39±6	38±6	2.5±2.8
17-18	5	34±5	37±5	36±4	35±5	1.3±6.5
MCVR (mmHg/mL/min)						
1.1	5	3.1±0.46	3.0±0.46	3.0±0.47	3.0±0.48	-2.1±2.7
3.4	5	3.0±0.49	3.2±0.76	3.4±7.4	3.0±0.48	0.7±2.2
11-13	5	3.1±0.57	2.9±0.53	2.8±0.49	3.0±0.56	-4.1±2.0
17-18	5	3.2±0.47	3.0±0.41	3.0±0.42	3.2±0.61	-2.8±5.5
R-R Interval (ms)						
1.1	5	661±72	588±66	581±51	669±75	1.8±5.5
3.4	5	637±52	634±54	625±58	632±61	-4.2±32.3
11-13	5	678±68	610±51	655±52	647±55	-3.3±6.1
17-18	5	748±96	617±58	623±63	608±61	-17.3±4.9
QT Interval (ms)						
1.1	5	239±7	220±7	229±5	229±14	-4.7±3.4
3.4	5	232±6	236±6	237±8	246±8	1.7±0.7
11-13	5	238±10	237±10	243±9	240±8	0.7±1.4
17-18	5	242±10	236±10	229±12	237±8	-2.0±2.5

CBF, Coronary blood flow; MCVR, Mean coronary vascular resistance.

Data are Mean±SEM.

¹Steady-state ranolazine plasma concentrations measured at 5-15 min after bolus and infusion of each of 4 doses (for doses, see METHODS).

Table 4Plasma Ranolazine Concentrations (μM) Following Intravenous Administration

Ranolazine	<u>In the Absence of Hexamethonium</u>		<u>In the Presence of Hexamethonium</u>	
	10 min	End of Infusion	10 min	End of Infusion
Low Dose	4 $\pm$ 0.2	5 $\pm$ 0.2	5 $\pm$ 0.2	5 $\pm$ 0.3
High Dose	11 $\pm$ 2	12 $\pm$ 2	13 $\pm$ 0.7	14 $\pm$ 0.8

Low Dose: 1.6 mg/kg bolus iv injection followed by 90 $\mu\text{g/kg/min}$ infusion for 30-40 min.High Dose: 3.6 mg/kg bolus iv injection followed by 200 $\mu\text{g/kg/min}$ infusion for 30-40 min.