Effect of Graded Heart Rate Reduction with Ivabradine on Myocardial Oxygen Consumption and Diastolic Time in Exercising Dogs

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Received September 9, 2003; accepted October 6, 2003

ABSTRACT

Lowering heart rate reduces myocardial oxygen consumption (MVO₂) and produces potent anti-ischemic effects. The development of selective heart rate-reducing agents represents an alternative approach to the use of β-blockers. Therefore, our goal was to establish the dose-response curve of the effects of ivabradine (If channel inhibitor) on MVO₂ and diastolic time. Seven conscious and chronically instrumented dogs were investigated during exercise at spontaneous and paced heart rate (250 beats/min) after administration of increasing doses of ivabradine (0.25, 0.5, and 1 mg/kg i.v.). During exercise, ivabradine dose dependently and significantly reduced the exercise-induced tachycardia (17, 21, and 32% at 0.25, 0.5, and 1 mg/kg, respectively, versus saline) without altering myocardial contractility nor mean ejection wall stress. A linear relationship between heart rate (HR) and MVO₂ was demonstrated (MVO₂ = 0.044 × HR − 1.4; r = 0.987). These effects of ivabradine on MVO₂ were abolished by atrial pacing. Similarly, ivabradine dose dependently increased diastolic time without altering the inverse and non-linear relationship between diastolic time and heart rate observed with saline. In conclusion, selective heart rate reduction with ivabradine dose dependently increases diastolic time and reduces MVO₂ with a linear relationship between heart rate and MVO₂. The lack of “on-off” pharmacological profile will predict the possibility of using a wide range of dose regimen.

Materials and Methods

The animal instrumentation and the experiments were performed in accordance with the official regulations of the French Ministry of Agriculture (approval no. A 94-043-12).

Instrumentation. Seven dogs (20–30 kg) were anesthetized with pentobarbital sodium (30 mg/kg i.v.), intubated, and ventilated with room air enriched with oxygen (30%). A left thoracotomy was performed.

This study was supported by a grant from the Fondation de France (2001-005170). P.C. was a recipient of the Société Francaise de Pharmacologie. Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

DOI: 10.1124/jpet.103.059717.

ABBREVIATIONS: MVO₂, myocardial oxygen consumption; LV dP/dt, first derivative of left ventricular pressure over time; HR, heart rate; DT, diastolic time.
formed through the 5th intercostal space using sterile technique. Fluid-filled Tygon catheters were placed in the descending thoracic aorta and the left atrium. Silastic catheters were implanted in the pulmonary artery and in the coronary sinus. A solid state pressure transducer (PTA; Konigsberg Instruments, Pasadena, CA) was introduced into the apex of the left ventricle. A flow probe (Transonic Systems, Ithaca, NY) was placed on the left circumflex coronary artery for continuous measurement of coronary blood flow. Piezoelectric ultrasonic dimension crystals were implanted 1) on opposed anterior and posterior endocardial surfaces of the left ventricle to measure left ventricular internal diameter, and 2) on opposed left ventricular endocardial and epicardial surfaces to measure wall thickness. Finally, stainless steel wires were sewn to the left atrial appendage for subsequent electrical pacing. All catheters and wires were exteriorized between the scapulae and the pneumothorax was evacuated. Cefazolin (1g i.v.) and gentamicin (40 mg i.v.) were administered before and during the first week after surgery. Buprenorphine (0.3 mg s.c.) was also administered during this period. The position of all catheters and crystals was confirmed at autopsy.

**Hemodynamic Measurements.** All hemodynamic data were continuously recorded, digitized, and analyzed using HEM version 3.2 software (Notocord Systems, Croissy sur Seine, France). Aortic and left atrial pressures were measured with a Statham P23ID strain gauge transducer (Statham Instruments, Oxnard, CA). Coronary blood flow was measured using a T206 blood flowmeter (Transonic Systems). Left ventricular pressure, internal diameter, and wall thickness were digitized at 500 Hz. Left ventricular pressure was calibrated in vitro with a mercury manometer and in vivo with the left atrial and aortic pressures. The maximal change in left ventricular pressure over time (LV dP/dt max) was computed from the left ventricular pressure signal. Left ventricular pressure signal was defined as the point of maximum negative LV dP/dt. Left ventricular end-systolic and end-diasstolic wall stresses were calculated with a cylindrical model as stress = \(1.36 \times (LVP \times ID/2h)\), where LVP is left ventricular pressure, ID is the internal short-axis diameter, and h is wall thickness, each of these parameters being measured at end-systole and end-diastole. The left ventricular peak systolic wall stress was computed as the maximal value of left ventricular wall stress during the ejection period. The integral of the systolic wall stress time-curve so-called mean ejection wall stress was calculated during the ejection period, i.e., from the maximum positive LV dP/dt to the maximum negative LV dP/dt. Percentage of wall thickening was defined as end-systolic minus end-diastolic thickness divided by end-diastolic thickness \(\times 100\).

**Left Ventricular Oxygen Consumption.** Measurement of oxygen content was made with a blood gas apparatus and a co-oximeter (BG III Synthesis, Albertville, MI). Oxygen delivery was calculated as the product of mean coronary blood flow and arterial oxygen content. Oxygen consumption was calculated as the product of mean coronary blood flow and the arteriovenous difference in oxygen content. Assumption was made that left circumflex coronary artery blood flow was proportional to the total left ventricular coronary flow and that the proportionality constant does not vary with exercise and/or drugs.

**Experimental Protocol.** The experiments were conducted 3 to 4 weeks after surgery when the dogs were healthy and apyretic. While the dogs were standing quietly on the treadmill, “baseline” parameters were recorded and blood samples were taken simultaneously from the aorta and the coronary sinus. A second set of measurements and blood samples collection was performed 15 min after the onset of drug administration, both at spontaneous heart rate (so-called “rest”) and during a sequence of 5 min of atrial pacing at a rate of 125 beats/min (so-called “rest paced”). Treadmill exercise (10 km/h, slope 13%, 10 min) was then started. The first 5 min of exercise was performed at spontaneous heart rate, with a set of measurements and blood sample collection being performed when a hemodynamic steady state was achieved (so-called “exercise”). The last 5 min of exercise was performed under atrial pacing at a rate of 250 beats/min (so-called “exercise-drug paced”), with a last set of measurements and blood sample collection being performed at the end of this stage.

**Drugs.** Each dog underwent four experimental sequences (saline, and 0.25, 0.5, and 1 mg/kg ivabradine), which were performed in random order 1 week apart. Ivabradine (Laboratoires Servier, Neuilly-sur-Seine, France) and saline were administered through the pulmonary artery catheter as an i.v. bolus. We have previously demonstrated that hemodynamic changes are reproducible when exercises are repeated (Berdeaux et al., 1994).

**Statistical Analysis.** All results are mean ± S.E.M. Statistical analysis was performed with two-way analysis of variance for repeated measures. When overall differences were detected, individual comparisons among drugs at each time point only were performed by paired Student’s t test with Bonferroni’s correction. The statistical analysis was performed using Statview 5.0 software (Abacus Concepts, Berkeley, CA). A value of \(p < 0.05\) was considered as statistically significant.

**Results**

**Hemodynamics.** As shown in Table 1, baseline hemodynamics values were not significantly different among the four sequences of the protocol, and none of these parameters was altered at rest after saline administration. Ivabradine dose dependently and significantly reduced heart rate (−16 and −21% at 0.5 and 1 mg/kg, respectively) and increased left ventricular end-diastolic wall stress. These effects were abolished by atrial pacing. Interestingly, ivabradine did not alter left ventricular mean ejection wall stress.

During exercise, the three doses of ivabradine dose dependently and significantly reduced the exercise-induced tachycardia (−17, −21, and −32% at 0.25, 0.5, and 1 mg/kg, respectively) and increased both left ventricular end-diastolic pressure and end-diastolic wall stress without changes in left ventricular mean ejection wall stress and LV dP/dt max. These effects were abolished by atrial pacing.

**Left Ventricular Myocardial Oxygen Consumption.** As shown in Table 2, neither saline nor ivabradine had any effect on resting coronary blood flow or myocardial oxygen balance at spontaneous and paced heart rates. Under saline, MVO2 during exercise increased 2.7-fold and coronary blood flow increased 1.9-fold from corresponding resting values at spontaneous heart rate. During exercise, ivabradine dose dependently and significantly decreased MVO2 versus saline (−7.6, −10.1, and −19% at 0.25, 0.5, and 1 mg/kg, respectively), and these effects were abolished by atrial pacing. Similar effects on coronary blood flow were observed. Values of hemoglobin were not significantly different among the four sequences (data not shown). As shown in Fig. 1, a linear relationship between heart rate (HR) and MVO2 was demonstrated (MVO2 = 0.044 × HR – 1.4; \(r = 0.987\)). In addition and as illustrated in Fig. 2, there were no significant differences among values of O2 delivery to O2 consumption ratio among sequences both at rest and during exercise at spontaneous heart rate. Finally, ivabradine significantly increased MVO2 per beat during exercise (4.2 ± 0.4 and 4.3 ± 0.4 ml of \(O2 \times 100/\text{beat}\) at 0.5 and 1 mg/kg, respectively, versus 3.6 ± 0.4 ml of \(O2 \times 100/\text{beat}\) for saline); this effect was abolished by atrial pacing.
Time Intervals. At rest, ivabradine dose dependently and significantly (0.5 and 1 mg/kg) increased both diastolic time and ejection time. These effects were abolished during atrial pacing (Table 2). As shown in Fig. 3, an inverse nonlinear relationship was demonstrated between HR and diastolic time (DT) when all data points were plotted, i.e., saline plus ivabradine. The relationship between these two parameters was not affected by ivabradine (DT = 884 e^{-0.0067 HR} for saline and DT = 901 e^{-0.0086 HR} for ivabradine).

Discussion

This study investigated the effects of graded heart rate reduction with ivabradine on myocardial oxygen consumption and diastolic perfusion time at rest and during exercise in chronically instrumented normal conscious dogs. Ivabradine is a selective I<sub>K1</sub> channel inhibitor (Thollon et al., 1994) that dose dependently and specifically decreases heart rate during rest and exercise (Simon et al., 1995; Monnet et al.,...
2001) without inducing any other significant hemodynamic alterations, in agreement with previous results. A linear relationship was observed between the reduction in heart rate provided by ivabradine and the decrease in MVO2. This demonstrates that in this context, one cannot define an optimal dose of ivabradine but rather a wide range of doses that can be used, depending on the level of heart rate and/or MVO2 reduction that needs to be achieved. Finally, heart rate reduction increased the diastolic time and ivabradine did not alter the intrinsic relationship between heart rate and diastolic time.

At all investigated doses, ivabradine did not affect either left ventricular contractility or mean ejection wall stress at rest and during exercise. It is also known that ivabradine does not affect the isovolumic relaxation (Colin et al., 2002). An increase in left ventricular filling pressure and in left ventricular end-diastolic wall stress was observed during exercise under ivabradine. This increase in left ventricular end-diastolic pressure was mainly related to the use of preload reserve because of the blunted heart rate response (Paulus et al., 1992). Indeed, when heart rate was corrected by atrial pacing, left ventricular end-diastolic pressure and wall stress were no longer different from saline. Therefore, the dose-dependent decreases in MVO2 with ivabradine were mainly related to the sole reduction in heart rate, confirming, if necessary, that this I$_f$ channel inhibitor is devoid of intrinsic effect on myocardial contractility.

Regarding the major reductions in heart rate, the related decreases in MVO2 induced by ivabradine seems not to be as great as expected. This could be explained by the fact that heart rate is not the sole determinant of myocardial oxygen demand (Braunwald, 1971). Furthermore, stroke volume is also one of the correlates of MVO2, as previously demonstrated by Rooke and Feigl (1982). This parameter was not measured in this study, but we previously demonstrated that ejection volume was increased after administration of ivabradine in a similar experimental model (Simon et al., 1995). We can hypothesize that part of the beneficial effects of reducing heart rate on MVO2 may be partially counteracted by an increase in stroke work. Indeed, MVO2 per beat was significantly increased with ivabradine. Nevertheless, we previously demonstrated that such reductions in heart rate were able to exert potent anti-ischemic and antistunning effects (Monnet et al., 2001). Finally, it seems that the effects of ivabradine on MVO2 depends upon the degree of activation of the autonomic nervous system, i.e., despite significant heart rate reduction with ivabradine, no effect on MVO2 was observed at rest. Indeed, the slope of the relationship between MVO2 and heart rate was below unity (0.044), which means that MVO2 will be reduced with important changes in heart rate.

In the present study, all changes in MVO2 were closely related to those of coronary blood flow. The O2 delivery to O2 consumption ratio was not significantly affected by ivabradine, demonstrating that the normal relationship between oxygen supply and oxygen demand was not modified at rest and during exercise by ivabradine. Indeed, we previously demonstrated that ivabradine does not alter coronary vaso-motion at rest and during exercise in normal dogs (Simon et al., 1995). Finally, because diastolic time is an important
determinant of myocardial oxygen supply, we investigated the effect of graded doses of ivabradine on this parameter. For this purpose, diastolic time was plotted against the corresponding heart rate at each stage of the protocol. The diastolic time-heart rate relationship demonstrated an inverse nonlinear pattern without any difference in this relationship between saline and ivabradine during exercise. This might be clinically relevant as Ferro et al. (1991b) previously indicated the favorable role of diastolic perfusion time in determining myocardial ischemia. This result also supports the fact that ivabradine is devoid of negative inotropism because it is known that reducing heart rate with β-blockers alters this relationship as a consequence of their negative inotropic effect (Ferro et al., 1991a).

The present study represents to our knowledge the first one investigating a full dose-response curve of the effects of a heart rate-reducing agent such as ivabradine on MVO₂. Our study clearly demonstrates a linear relationship between this parameter and the level of heart rate reduction. Because ivabradine is devoid of any other hemodynamic properties, i.e., inotropic, lusitropic or vasoactive effects, its administration only acts on heart rate (Simon et al., 1995; Colin et al., 2002). Interestingly, the effects observed on MVO₂ with increasing doses of ivabradine are progressive and a significant reduction in MVO₂ can be achieved even with small doses at exercise. In another words, ivabradine can exert its pharmacological properties dose dependently on heart rate and therefore on MVO₂, within a wide range of doses. This might be clinically relevant because controlling heart rate and diastolic time are major goals to achieve in the treatment of ischemic heart disease. The unique pharmacological profile of ivabradine allows a precise dose adjustment because one can predict the decrease in heart rate and hence in MVO₂ induced by the drug.

In conclusion, ivabradine dose dependently reduces MVO₂ during exercise, demonstrating a linear relationship between heart rate and MVO₂ reductions without any other hemodynamic effects. It also dose dependently increases the diastolic perfusion time. These pharmacological properties of ivabradine have important clinical implications because its action on both heart rate and diastolic perfusion time is of major importance in the cardioprotective effects of ivabradine. Furthermore, the lack of “off” pharmacological profile will predict the possibility of using a wide range of dose regimen.

Acknowledgments
We thank Drs. F. Mahlberg, J. P. Vilaine, and G. Lerebours (all from Laboratoires Servier) for fruitful discussions during the elaboration of this manuscript. We are also greatly indebted to Alain Bizé and Stéphane Bloquet for technical assistance.

References