I_f inhibition in the atrioventricular node by ivabradine causes rate-dependent slowing of conduction and reduces ventricular rate during atrial fibrillation

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BACKGROUND I_f channels are functionally expressed in atrioventricular (AV) nodal tissue.

OBJECTIVE The purpose of this study was to address whether the prototypical I_f inhibitor, ivabradine, at clinically safe concentrations can slow AV node conduction to reduce ventricular rate (VR) during atrial fibrillation (AF).

METHODS Effects of ivabradine (0.1 mg/kg IV bolus) were studied in an anesthetized Yorkshire pig (N = 7) model of AF and in isolated guinea pig hearts (N = 7).

RESULTS Ivabradine reduced heart rate (P = .0001) without affecting mean arterial pressure during sinus rhythm. The agent lengthened PR intervals in a rate-dependent manner (P = .0009) by 14 ± 2.7 ms (P = .003) and 25 ± 3.0 ms (P = .0004) and increased atrial-His (A-H) intervals in a rate-dependent manner (P = .020) by 10 ± 1.7 ms and 17 ± 2.8 ms during pacing at 130 and 180 bpm, respectively (both P = .0008). Similar rate-dependent effects were observed in isolated guinea pig hearts. Ivabradine slowed VR during AF from 240 ± 21 bpm to 211 ± 25 bpm (P = .041). The ivabradine-induced increase in A-H interval was inversely correlated with VR (r = −0.85, P = .03, at 130 bpm; r = −0.95, P = .003, at 180 bpm). QT and HV intervals, AF dominant frequency (8.5 ± 0.9 to 8.7 ± 1.1 Hz, P = NS), mean arterial pressure, and left ventricular dP/dt (1672 ± 222 to 1889 ± 229 mm Hg/s, P = NS) during AF were unaffected.

CONCLUSION Ivabradine's rate-dependent increase in A-H interval is highly correlated with VR during AF. As dominant frequency was unaltered, AV node conduction slowing during high nodal activation rates appears to be the main mechanism of ivabradine's VR reduction. I_f inhibition in the AV node may provide a promising target to slow VR during AF without depression in contractility.

KEYWORDS I_f current; Atrioventricular node; Ivabradine; Ventricular rate; Atrial fibrillation

ABBREVIATIONS **ACh** = acetylcholine; **AF** = atrial fibrillation; **A-H** = atrial-His; **AV** = atrioventricular; **ECG** = electrocardiogram; **HCN** = hyperpolarization-activated, cyclic nucleotide-gated; **H-V** = His-ventricular; **LV** = left ventricle; **MAP** = mean arterial pressure; **mRNA** = messenger RNA; **SA** = sinoatrial; **S-H** = stimulus to His

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Introduction

There is increasing evidence that the funny current, I_f, may play a role in regulating conduction through the atrioventricular (AV) node. This view is based on the observations that I_f is functionally expressed in nodal tissue.[1–4] Zatebradine, a relatively selective I_f inhibitor, causes a dose-dependent increase in atrial-His (A-H) interval and a decrease in AV junctional rate following crushing of the sinoatrial (SA) node in a canine model.[5] In a double-blind placebo-controlled study in humans, zatebradine was shown to affect several AV node functions, including prolongation of A-H interval and increase in AV node effective refractory period and Wenckebach cycle length.[6]

The actions of the prototypical I_f inhibitor, ivabradine, have been thought to be confined to the sinoatrial (SA) node. During spontaneous rhythm, electrophysiologic studies have not revealed AV node prolongation, even with a relatively high dose of 0.2 mg/kg IV.[7] However, the sinus rate–slowing

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effect of ivabradine,[8] which results in a rate-dependent shortening of A-H interval, may have masked ivabradine's potential to prolong the A-H interval. In fact, ivabradine causes a strong rate-dependent effect on sinus rate slowing, as this agent gains access to intracellular I_f channels during the open state.[4,9] We recently demonstrated that dronedarone, which has been shown clinically to reduce heart rate during sinus rhythm and to slow ventricular rate during atrial fibrillation (AF),[10–13] may exert these effects largely through I_f inhibition.[14–16]

The goal of the present study was to determine whether by slowing conduction through the AV node, ivabradine prolongs the A-H interval in a rate-dependent manner both in intact Yorkshire pigs and in isolated guinea pig hearts. Also, we examined whether a slowing in AV node conduction would result in a clinically relevant reduction in ventricular rate during AF. Because ivabradine as well as other I_f inhibitors do not exhibit negative inotropic actions,[5,14,15] demonstration of a reduction in ventricular rate during AF by ivabradine could offer an advantage over certain conventional nodal agents such as calcium channel and beta-adrenergic receptor blocking agents.

Methods

Porcine model experimental preparation

This study conformed to the *Position of the American Heart Association on Research Animal Use* as well as to the Declaration of Helsinki. The protocol was approved by the animal use committee of Beth Israel Deaconess Medical Center. Experiments were carried out in male Yorkshire pigs ($N = 7$) weighing 36.7 ± 1.2 kg (mean $\pm$ SEM). The animals were preanesthetized with telazol (4.7 mg/kg intramuscularly) and then anesthetized with alpha-chloralose (100 mg/kg IV bolus followed by 40 mg/kg/h IV continuous infusion). The animals were intubated, and ventilation was maintained between 10 and 16 breaths/min with volumes between 400 and 500 mL. Vital signs including heart rate and oxygen saturation were continuously monitored. Core body temperature was maintained constant at $99.8° \pm 0.4$ºF to $99.2° \pm 0.8$ºF using a heating pad.

Access to the arteries and veins was achieved using the Seldinger technique to introduce 7Fr sheaths. Mean arterial blood pressure (MAP) was continuously calculated from signals recorded from a femoral arterial sheath, and intravenous fluids were administered through the ear vein. The electrocardiogram (ECG) was recorded using a Prucka CardioLab workstation (GE Medical Systems, Milwaukee, WI) from atrial and ventricular sites.

Ivabradine was administered as a 0.1 mg/kg IV bolus infused over 5 minutes via a 7Fr sheath inserted into the right femoral vein. This dose was selected because it corresponds to the lowest intravenous dose tested clinically.[17,18]

Plasma level determination

Blood samples were collected in sodium heparin tubes at 0, 15, 30, 45, and 60 minutes after ivabradine bolus administration. The samples were centrifuged and frozen at –80°C until drug level determination was performed. Concentrations of ivabradine in plasma were determined at Gilead Sciences, Inc. (Foster City, CA). Plasma was analyzed using a high-performance liquid chromatography–tandem mass spectrometric assay (LC/MS/MS). Ivabradine plasma levels were quantified by LCQuan v2.5.5 (ThermoFisher Scientific, Asheville NC). The quantification limit was 4 nM (1.87 ng/mL). The dynamic range of quantification was 4 to 10,000 nM (1.87–4686 ng/mL).

Cardiac catheterization

A nonsteerable, quadripolar or decapolar electrode catheter (Bard Electrophysiology, Lowell, MA) was placed in the right atrium via the femoral vein and a quadripolar electrode catheter in the right ventricle via the jugular vein (Figure 1). A tripolar electrode catheter was placed in the noncoronary cusp of the aorta through the left carotid artery to record the His-bundle electrogram. A pigtail catheter was inserted in the left ventricle (LV) via the left femoral artery to record LV pressure and to calculate contractility (LV dP/dt). Transatrial access, as previously described,[19] was used to deliver acetylcholine (ACh) into the pericardial space. Specifically, a small puncture was made in the right atrial appendage with the stiff end of a coronary angioplasty guidewire (0.014-inch Wizdom guidewire, Cordis, Miami, FL) placed within the lumen of a soft infusion catheter (0.038-inch SOS straight-tip, open-ended angiographic guidewire, Bard Electrophysiology). This wire-within-wire system was advanced as a unit into an 8Fr multipurpose guide catheter (MP2, Boston Scientific, Boston, MA) previously positioned in the right atrial appendage via a femoral vein under fluoroscopic guidance and into the pericardial space. The infusion catheter was left in the pericardial space for delivery of ACh, and the inner guidewire was removed. Conformation of the infusion catheter on fluoroscopy to the curvature of the heart verified its location within the pericardial space. All of the pericardial fluid was then aspirated with the infusion catheter. The low pericardial fluid hematocrit ($<2\%$) following transatrial access indicates minimum trauma from the atrial puncture.[19] The presence of acetylcholinesterase can rapidly degrade ACh and prevent a sustained response to this neurotransmitter.

Electrical testing

A Bloom stimulator (Bloom Associates, Reading, PA) was used to deliver constant-current rectangular stimuli for fixed-rate pacing as well as for delivering premature stimuli.

Porcine model protocol for AF induction and analysis

AF was defined as an irregular atrial rhythm with an average cycle length <150 ms at all atrial sites, whereas atrial flutter was defined as a regular atrial rhythm with fixed cycle length >150 ms at all atrial sites. After baseline electrical testing was performed, AF initiation was attempted by decremental burst pacing at a cycle length of 200 ms at each atrial site down to loss of 1:1 capture. ACh (1 mL of 100 mM solution) along with a 1-mL saline flush was injected into the pericardial space