Atrial Antifibrillatory Effects of Structurally Distinct $I_{Kur}$ Blockers 3-[(Dimethylamino)methyl]-6-methoxy-2-methyl-4-phenylisoquinolin-1(2H)-one and 2-Phenyl-1,1-dipyridin-3-yl-2-pyrrolidin-1-yl-ethanol in Dogs with Underlying Heart Failure

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ABSTRACT

Drug discovery efforts have focused recently on atrial-selective targets, including the Kv1.5 channel, which underlies the ultrarapid delayed rectifier current, $I_{Kur}$, to develop novel treatments for atrial fibrillation (AF). Two structurally distinct compounds, a triarylethanolamine TAEA and an isoquinolinone 3-[(dimethylamino)methyl]-6-methoxy-2-methyl-4-phenylisoquinolin-1(2H)-one (ISQ-1), blocked $I_{Kur}$ in Chinese hamster ovary cells expressing human Kv1.5 with IC$_{50}$ values of 238 and 324 nM, respectively. In anesthetized dogs, i.v. infusions of TAEA and ISQ-1 elicited comparable 16% increases in atrial refractory period, with no effect on ventricular refractory period or QTc interval. Plasma concentrations at end infusion for TAEA and ISQ-1 were 58±23.6 and 330.3±43.5 nM, respectively. The abilities of TAEA and ISQ-1 to terminate AF, with comparison to the rapidly activating component of delayed rectifier potassium current blocker (+)-N-[6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl]-3,4-dihydro-4(R)-hydroxyspiro(2H-1-benzopyran-2,4′-piperidin)-6-yl)methanesulfonamide] monohydrochloride (MK-499) and the class IC 1-[2-[2-hydroxy-3-(propylamino)propoxy]phenyl]-3-phenyl-1-propanone (propafenone), were assessed in conscious dogs with heart failure and inducible AF (entry criterion). All test agents administered in i.v. bolus regimens terminated AF in at least half of animals tested; conversely no agent was universally effective. MK-499, ISQ-1, TAEA, and propafenone terminated AF in five of six, four of seven, four of six, and five of six animals at plasma concentrations of 32.6±18.7, 817±274, 714±622, and 816±240 nM, respectively. Directed cardiac electrophysiologic studies in anesthetized dogs using i.v. bolus (consistent with AF studies) plus infusion regimens with TAEA and ISQ-1 demonstrated significant increases in atrial refractory period (12–15%), A-H and P-A intervals, but no effects on ventricular refractory period, H-V, and HEG intervals. The demonstration of AF termination with TAEA and ISQ-1 in the dog heart failure model extends the profile of antiarrhythmic efficacy of Kv1.5 blockade.

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, associated with significant cardiovascular morbidity and mortality, and is increasing dramatically in prevalence, hospitalization rate, and economic burden. Presently available antiarrhythmic therapy for AF is inadequate and limited by the occurrence of adverse effects, including ventricular proarrhythmia (Valderrama et al., 2005; Waldo, 2006). In an attempt to identify safer and more effective agents for the treatment of AF, drug discovery efforts have focused on atrial-selective agents, including blockers of the Kv1.5 potassium channel, which underlies the ultrarapid delayed rectifier current, $I_{Kur}$. (Nattel and Carlsson, 2006).

The recent development of animal models in which AF occurs in the setting of underlying cardiac pathology has

ABBREVIATIONS: AF, atrial fibrillation; $I_{Kur}$, ultrarapid delayed rectifier potassium current; TAEA, 2-phenyl-1,1-dipyridin-3-yl-2-pyrrolidin-1-yl-ethanol; ISO-1, 3-[(dimethylamino)methyl]-6-methoxy-2-methyl-4-phenylisoquinolin-1(2H)-one; $I_{op}$, rapidly activating component of delayed rectifier potassium current; MK-499, (+)-N-[6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl]-3,4-dihydro-4(R)-hydroxyspiro(2H-1-benzopyran-2,4′-piperidin)-6-yl)methanesulfonamide] monohydrochloride; hERG, human ether-a-go-go-related gene; bpm, beats per minute; AET, atrial excitation threshold; ARP, atrial refractory period; VET, ventricular excitation threshold; VRP, ventricular refractory period; EP, electrophysiologic; LV, left ventricular; RV, right ventricular; PEG, polyethylene glycol; ANOVA, analysis of variance; HR, heart rate; NIP-142, (3R*,4S*)-4-cyclopropylamino-3,4-dihydro-2,2-dimethyl-6-(4-methoxyphenylacetylaminio)-7-nitro-2H-1-benzopyran-3-ol; DPO-1, 2-isopropyl-5-methylcyclohexyl diphenylphosphine oxide; AVE-0118, 2′-[2-(4-methoxyphenyl)-acetylaminio]-methyl-biphenyl-2-carboxylic acid (2-phenyl-3-yl-ethyl)-amide; RSD1235, (1R,2R)-2-[(3R)-hydroxypropyrroldinyl]-1-(3,4-dimethoxyphenethoxy)-cyclohexane.

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facilitated the study of underlying electrophysiological mechanisms of this arrhythmia and the assessment of novel antiarrhythmic agents (Nattel et al., 2005). One such animal model is the dog in which rapid ventricular pacing induces ventricular dysfunction, leading to atrial remodeling and the ability to sustain AF (Li et al., 1999, 2000b; Ryu et al., 2005). The present study used the dog heart failure AF model to demonstrate the atrial antiarrhythmic efficacies of two structurally distinct Kv1.5 blockers, a triarylethanolamine, 2-phenyl-1,1-dipyrindin-3-yl-2-pyrrolidin-1-yl-ethanol (TAEA) and an isoquinolinone, ISQ-1 (Trotter et al., 2006; Regan et al., 2007) (Fig. 1). For comparison, the class IC sodium channel blocker 1-[2-[2-hydroxy-3-(propylamino)-propoxy]phenyl]-3-phenyl-1-propanone (propafenone) and the prototype delayed rectifier Ik1 blocker MK-499 (Lynch et al., 1994) were assessed concurrently in this model.

Materials and Methods

All procedures related to the use of animals in these studies were reviewed and approved by the Institutional Animal Care and Use Committee at Merck Research Laboratories at West Point, PA, and they conform with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

In Vitro Assessment of Kv1.5 Blockade. The in vitro pharmacology of Kv1.5 blockers was determined using high-throughput patch-clamp electrophysiology. Currents were recorded at room temperature (21–23°C) using the IonWorks HT system (Molecular Devices, Sunnyvale, CA). Membrane currents were amplified and sampled at 100-μs intervals (10 kHz). Stimulation protocols and data acquisition were carried out using a microcomputer (Dell Pentium 4), running Ionworks HT software and hardware. Leak subtraction was performed in all experiments by applying one hyperpolarizing pre-pulse before the test pulses (P/1 subtraction protocol).

Cell Preparation for Electrophysiology. Kv1.5 was expressed in Chinese hamster ovary cells using the Flp-In system (Invitrogen, Carlsbad, CA). Cells were grown to 60 to 100% confluence in a T75 flask. Cells were lifted by removing the growth media and incubating with 1.5 ml of warmed (37°C) Versene (Invitrogen) for 5 to 7 min. Lifted cells were suspended in 10 ml of phosphate-buffered saline (Invitrogen). Cell suspension was then placed into a 10-ml centrifuge tube and centrifuged for 4 min at approximately 50g. After centrifugation, the supernatant was removed, and the cell pellet was resuspended in 5 ml of phosphate-buffered saline. Cells were then added to the cell suspension boat in IonWorks HT.

Solution and Drugs. The intracellular solution contained 140 mM KCl, 1 mM MgCl2, 1 mM EGTA, and 20 mM HEPES, adjusted to pH 7.3. The external solution was Dulbecco’s phosphate-buffered saline (Invitrogon) and contained 0.90 mM CaCl2, 2.67 mM KCl, 1.47 mM KPO4, 0.50 mM MgCl2, 138 mM NaCl, and 8.10 mM NaPO4, pH 7.4. Compounds were synthesized by the medicinal chemistry department at Merck Research Laboratories. All compounds were prepared as 10 mM stock solution in dimethyl sulfoxide (maximum final concentration 1% vol/vol). In preliminary experiments, we confirmed that <1% dimethyl sulfoxide had no effect on the parameters under study.

Patch-Clamp Protocol. A protocol was developed for automated voltage-clamping of cells in 384-well PatchPlate. The single cell recording was performed in each well of the PatchPlate (Molecular Devices). Upon membrane depolarization, the Kv1.5 channel is activated rapidly, and an outward current is measured. Current evoked by a voltage-ramp protocol revealed that the channel activation potential was approximately −20 mV, consistent with what has been reported for the native Kv1.5 (Wang et al., 1993). The following protocol was used to measure Kv1.5 channel activity and pharmacology. The holding potential was −80 mV. Outward Kv1.5 currents were elicited by depolarizing the membrane potential to +40 mV for 100 ms. A train of depolarizing steps was applied at 5 Hz for 8 s. The average current elicited by the last +40-mV step in the train was 4.4 ± 1 nA (n = 1100), and most of these currents were >3 nA. The homogenous expression of Kv1.5 using Flp-In system allowed for rapid analysis of test agent pharmacology using electrophysiological recordings. To measure the effect of compounds, the current amplitude (elicited by the last +40-mV step in the train) was measured after cells were preincubated with tested compound, and it was compared with current amplitude before addition of the compound. The effect of a single concentration of a compound was measured in a single cell. Dose-dependent inhibition of Kv1.5 current with TAEA and ISQ-1 was determined from the culmination of data from multiple cells. TAEA and ISQ-1 did not display voltage dependence of inhibition of Kv1.5 current.

Selectivity. Activity against the hERG channel, which underlies IKr, was assessed using an in vitro MK-499 binding assay in human embryonic kidney-293 cells expressing hERG (Wang et al., 2003). Selectivity against a broad panel of receptors, ion channels, and transporters was determined in in vitro radioligand binding screening assays (MDS Pharma Services, Taipei, Taiwan).

Acute Cardiac Electrophysiologic Studies in Dogs. The surgical preparation and methods for the measurement of cardiac electrophysiologic parameters in anesthetized dog were as described previously (Regan et al., 2007). Briefly, mongrel dogs [male or female; 6.8–12.6 kg; 9.2 ± 0.4 kg (mean ± S.E.)] were anesthetized with 100 mg/kg i.v. α-chloralose and 5 mg/kg i.v. sodium pentobarbital (baseline sinus heart rate 127 ± 5 bpm). A continuous infusion of 10 mg/kg/h i.v. α-chloralose was used to maintain anesthesia over the time course of the study. The animals were intubated and ventilated with room air. The right femoral artery and vein were cannulated for the measurement of mean arterial pressure and for maintenance anesthesia, respectively. The left femoral vein was cannulated for test agent administration. A left thoracotomy was performed, and the pericardium was incised to expose the heart. A bipolar epicardial electrode was sutured to the right atrium for pacing, and a quadripolar epicardial electrode was sutured to the left atrium for the recording of atrial electrograms and for the determination of atrial excitation threshold (AET) and atrial refractory period (ARP). A bipolar plunge electrode was sutured to the posterolateral wall of the left ventricle for the determination of ventricular excitation threshold (VET) and ventricular refractory period (VRP). Refractory periods were determined by extrastimulus technique at a rate of 150 beats/min (400-ms cycle length) at 2 times excitation threshold.
threshold. Pin electrodes were attached for the recording of lead II ECG, including rate-corrected QTc interval (QT in milliseconds/√R-R in seconds).

Dog cardiac electrophysiologic (EP) studies used within-group repeat EP testing during continuous i.v. infusions of test agent or vehicle in volume- and timing-matched controls. Following a 30-min equilibration period, animals were randomized to 60 min continuous i.v. infusions of vehicle or test agent TAEA at 0.7 μg/kg/min (n = 6 each). An identical dosing paradigm had been used previously to characterize the cardiac EP effects of ISQ-1 at 7.0 μg/kg/min (Regan et al., 2007), with pharmacokinetic modeling having been performed to guide dose selection for these agents. Sterile water containing 25% hydroxypropyl-β-cyclodextrin, 20 ml administered over 60 min, served as the vehicle for 60-min continuous i.v. infusion studies. Studies with TAEA and ISQ-1 used separate temporally matched vehicle control groups. This aqueous vehicle has been used previously in cardiac EP studies in this laboratory for the conduct of longer term intravenous infusion studies, and it has no inherent effects on cardiac EP parameters in dog. Heart rate, mean arterial pressure, cardiac EP parameters, and ECG intervals, including QTc interval, were determined before initiation of treatment (i.e., pre-treatment baseline) and at 15, 30, 45, and 60 min of continuous i.v. treatment infusion. Blood samples also were obtained at baseline and at 15, 30, 45, and 60 min of treatment for determination of plasma concentration of test agents.

AF Termination Studies in Dogs with Chronic Rapid Right Ventricular Pacing. Seven mongrel dogs (male or female; 20.5–25.0 kg; 21.8 ± 0.7 kg at time of initiation of cardiac EP and AF induction testing) were anesthetized with thiopental at 15 to 30 mg/kg i.v., followed by intubation and general anesthesia with isoflurane (1.5–2.0 volume % in oxygen). Using sterile surgical technique, a left thoracotomy was performed at the fifth intercostal space, and the pericardium was incised to expose the heart. A bipolar epicardial electrode was sutured to the right atrium for pacing, and a quadrupolar epicardial electrode was sutured to the left atrium for the recording of atrial electrograms and for the determination of AET and ARP. Bipolar plunge electrodes were sutured to the right and left ventricles for rapid ventricular pacing to produce heart failure and for the determination of VET and VRP, respectively. A solid state miniature pressure gauge (Königsberg, Pasadena, CA) was implanted into the left ventricular (LV) cavity for measurement of LV systolic pressure and rate of change of LV pressure (LV dP/dt). Electrode leads and the LV pressure gauge cable were tunneled subcutaneously and exterriorized dorsally via titanium skin buttons. At the time of surgical preparation, animals also were instrumented with an iliac arterial catheter vascular access port for the measurement of blood pressure. Following surgical instrumentation, the animals were allowed to recover from anesthesia.

Rapid right ventricular (RV) pacing at a rate of 240 bpm was initiated at 7 to 14 days after surgical preparation, and it was typically reduced after 2 to 3 weeks of pacing to rates ranging from 210 to 230 bpm based on clinical assessment. Continuous RV pacing was suspended temporarily during the conduct of cardiac EP and AF induction studies. Animals were studied while lying in right lateral recumbency either fully conscious or, occasionally if necessary, in the setting of very mild sedation (0.25 mg/kg i.m. xylazine, administered before baseline, pre-AF induction measures). Baseline heart rate, mean arterial pressure, ECG intervals, AET, ARP, VET, and VRP were determined as described in the preceding section. Immediately following baseline EP measures, AF induction was attempted by burst right atrial pacing (10 Hz; 1- to 10-s duration). Baseline-inducible AF was the entry criterion for study. When sustained AF (defined as duration ≥10 min) was induced, pharmacological termination was assessed using one of the following treatment regimens: MK-499 (0.001, 0.003, 0.01, and 0.03 mg/kg i.v.; ISQ-1 (0.03, 0.1, 0.3, and 1.0 mg/kg i.v.); TAEA (0.01, 0.03, 0.1, and 0.3 mg/kg i.v.); propafenone (0.01, 0.03, 0.1, and 0.3 mg/kg i.v.); or volume- and timing-matched vehicle. Animals received four increasing doses (or matched volume of vehicle) of one treatment on a given study day, with the doses administered as slow (30-s) i.v. boluses at 5-min intervals. A 15-min observation period followed the last i.v. dose. Test agents were studied in crossover manner. In animals in which AF terminated during treatment, a blood sample was drawn upon termination for determination of plasma concentration of test agent, and post-termination heart rate, mean arterial pressure, ECG intervals, and EP parameters were determined approximately 2 min following termination. Sterile 40% PEG-200/60% D5W, 10 ml per dose, served as the vehicle for all test agents. Animals were tested with different test agents no earlier than 2 days (≥5 days) for test agents) following the preceding test day. All seven animals entered into the study were administered volume- and timing-matched vehicle after the induction of persistent AF, whereas MK-499, ISQ-1, TAEA, and propafenone were assessed in either six or seven of the animals after the induction of sustained AF.

Directed Acute Cardiac Electrophysiologic Studies in Dogs Using Intravenous Bolus (Simulating AF Termination Study Administration) Plus Infusion Dosing. Following the conduct of AF termination studies, directed acute cardiac EP studies were conducted in anesthetized dogs using i.v. bolus dosing regimens of TAEA and ISQ-1 (simulating administration of these test agents in AF termination studies) plus continuous i.v. infusions of these test agents (simulating the initial acute cardiac EP studies described above), with the continuous infusions intended to maintain plasma test agent concentrations. Male or female mongrel dogs (n = 9; 7.1–10.0 kg; 8.6 ± 0.3 kg) were surgically prepared as described above in the initial acute cardiac EP studies with the following specific placement of electrodes: a bipolar epicardial electrode was sutured to the right atrium for atrial pacing; a bipolar epicardial electrode was sutured to the left atrium for local atrial electrogram recording; two bipolar plunge electrodes were sutured to the anterior wall of the left ventricle for pacing and for local ventricular electrogram recording; and a bipolar recording catheter (7 French) was advanced down the right carotid artery to the root of the aorta and positioned for the recording of His bundle conduction times (A-H and H-V intervals). Following a 30-min equilibration period, the following cardiac EP parameters were measured at a constant pacing rate of 150 bpm (400-ms cycle length) before treatment (baseline) and after test agent administration: P-A interval, an index of atrial conduction determined by measurement of the interval from the onset of the lead II P-wave to the onset of deflection of a fixed left atrial bipolar electrode; A-H interval, an index of atrioventricular nodal conduction determined by measurement of the interval from the earliest rapid deflection of the atrial electrogram (A) to the onset of the His (H) deflection of the His bundle recording; H-V interval, an index of His-Purkinje ventricular conduction time determined by measurement of the interval from the onset of the His (H) deflection to the earliest onset of ventricular activation recorded from the ventricular electrogram (V) of the His bundle recording; HEG interval, an index of ventricular conduction time determined by measurement of the interval from the onset of the His (H) deflection to peak deflection of a fixed left ventricular bipolar electrode; and ARP and VRP determined as described above in the initial acute cardiac EP studies.

Three groups of animals (n = 3 each) were administered TAEA (bolus i.v. 0.01, 0.03, 0.1, and 0.3 mg/kg) plus an underlying continuous i.v. infusion of 0.7 μg/kg/min; ISQ-1 (bolus i.v. 0.03, 0.1, 0.3, 1.0 mg/kg) plus an underlying continuous i.v. infusion of 7.0 μg/kg/min; or volume- and timing-matched vehicle. Bolus and infusion doses of TAEA and ISQ-1 in these directed cardiac EP studies were identical to those used in the AF termination and initial cardiac EP profiling studies described above. Bolus i.v. doses of test agent were administered as slow (30-s) i.v. boluses at 5-min intervals, and sterile 40% PEG-200/60% D5W, 10 ml per dose, served as the vehicle for the test agents as per the AF termination studies. Repeat cardiac EP testing was conducted immediately after each bolus dose of test agent or vehicle, with blood samples for determination of plasma concentra-
tion of test agents drawn at approximately 2 min after each bolus dose during the continuous i.v. test agent infusion.

Statistical Analyses. All data are expressed as mean ± S.E.M. For acute dog cardiac electrophysiologic studies, which used within group repeated EP testing during either continuous i.v. infusion of test agent or vehicle or combined i.v. bolus plus infusion of test agent or vehicle, the effects of test agent were analyzed using a two-way analysis of variance (ANOVA), including a within group repeated measures and a between group (test agent versus vehicle) comparison to detect test agent-specific changes differing significantly from the vehicle profile. If a significant test agent-specific change was indicated, a within-test agent treatment group repeated measures ANOVA followed by a post hoc Fisher’s protected least significant difference test was used to identify statistically significant changes from baseline values. For studies in the dog heart failure AF model, within group baseline versus post-treatment, post-AF termination comparisons were performed using a paired Student’s t test. Post-treatment values were derived at 2 min post-AF termination following the dose of test agent terminating AF in each individual animal. In all studies, among group comparisons of baseline values were conducted using a one-way analysis of variance. All statistical comparisons were performed using absolute data, whereas the effects of test agents on select parameters were expressed graphically as percentage change from pretreatment baseline value to normalize comparisons for differences in baseline values among different parameters.

Results

In Vitro Assessment of Kv1.5 Blockade. IC50 values for block of Kv1.5 current for TAEA and ISQ-1 were 238 and 324 nM, respectively (Fig. 2). In a hERG binding assay, TAEA and ISQ-1 displayed IC50 values of >30 and 15 μM, respectively. In a broad panel of radioligand binding screens, TAEA and ISQ-1 assayed at a test concentration of 10 μM each displayed activities of ≤20% versus a wide spectrum of receptor subtypes (including adrenergic, muscarinic, 5-hydroxytryptamine, dopamine, histamine, glutamate, and prostanoid), ion channels (L-type calcium, benzo diazepine, dihydropyridine, phenylalkylamine; N-type calcium, SKCa, K, KATP, site 2 sodium) and transporters (including monoamine, norepinephrine, and dopamine).

Acute Cardiac Electrophysiologic Studies in Dogs. In anesthetized dogs, the 60-min continuous i.v. infusion of 0.7 μg/kg/min TAEA elicited a significant maximal 16% increase in ARP, with no concomitant change in VRP or ECG QTc interval (Fig. 3). There were no significant effects of TAEA infusion on heart rate, mean arterial pressure, or ECG P-R and QRS interval (data not shown). The plasma concentration of TAEA determined at the end of the 60-min infusion was 58.5 ± 23.6 nM. The cardiac electrophysiologic effects of ISQ-1 administered as a 60-min continuous i.v. infusion of 7.0 μg/kg/min have been characterized in the same preparation, with a similar selective increase in ARP observed (Fig. 4) (Regan et al., 2007). The plasma concentration of ISQ-1 determined at the end of the 60-min infusion was 330.3 ± 43.5 nM. Consistent with previous lab experience, the aqueous 25% hydroxypropyl-β-cyclodextrin-containing vehicle used in these studies produced no effects on ARP, VRP, or QTc (Figs. 3 and 4).

AF Termination Studies in Dogs with Chronic Rapid Right Ventricular Pacing. Cardiac EP, AF induction, and pharmacological termination studies were initiated in seven conscious dogs at 24 to 44 (34.3 ± 2.9) days after the start of continuous RV pacing (i.e., a minimum of 4–5 weeks after surgical anesthesia and instrumentation). Serial assessment of active test agents and vehicle for AF termination occurred over a span of 14 to 27 (19.6 ± 1.7) days after the initiation of testing. Compared with pre-RV pacing baselines, changes of −20 and −12% in LV systolic pressure, +67 and +60% in LV end diastolic pressure, and −38 and −25% in LV dP/dt were observed at initiation and completion, respec-

Fig. 2. Block of Kv1.5 channel currents by TAEA and ISQ-1. Currents recorded from Kv1.5 under control conditions (A) and in the presence of 2400 nM ISQ-1 (B). Complete concentration response curves for block of Kv1.5 currents by TAEA (C) and ISQ-1 (D). The calculated IC50 value for TAEA and ISQ-1 block of Kv1.5 currents was 238 ± 5 and 324 ± 8 nM, respectively.

Fig. 3. Effects of continuous 60 min i.v. dose infusions of TAEA, 0.7 μg/kg/min for 60 min, or vehicle (sterile water containing 25% hydroxypropyl-β-cyclodextrin) on ARP, VRP, and ECG rate-corrected QTc interval in anesthetized dogs. Data are mean ± S.E.M., with n = 4 to 6. *p < 0.05; †p < 0.01 determined by using a two-way ANOVA, including a within-group repeated measures and a between-group (test agent versus vehicle) comparison to detect test agent-specific changes differing significantly from the vehicle profile. If a significant test agent-specific change was indicated, a within-test agent treatment group repeated measures ANOVA followed by a post hoc Fisher’s protected least significant difference test was used to identify statistically significant changes from baseline values. Baseline values for ARP, VRP, and QTc in dog cardiac electrophysiologic studies ranged from 104.3 ± 3.9 to 114.0 ± 6.4, 152.0 ± 6.2 to 160.3 ± 2.3, and 314.5 ± 10.3 to 350.8 ± 9.4 ms, respectively, and they did not differ significantly among treatment groups.
Baseline-inducible AF was required for animal entry into study. All seven animals tested responded reproducibly to right atrial burst pacing with sustained, i.e., ≥10 min continuous AF before test agent or vehicle administration. Administration of vehicle, four i.v. boluses at 5-min intervals commencing after 10 min of AF and with a 15-min observation period following the final administration, failed to terminate AF in all seven animals (representative study, Fig. 5). Therefore, AF of at least 40-min sustained duration was induced and maintained during vehicle testing in all seven animals entered into study. Accordingly, AF termination following test agent administration during this time frame of study was considered a response to active test agent as opposed to a stochastic event.

Administration of MK-499 (0.001, 0.003, 0.01, and 0.03 mg/kg i.v.), ISQ-1 (0.03, 0.1, 0.3, and 1.0 mg/kg i.v.), TAEA (0.01, 0.03, 0.1, and 0.3 mg/kg i.v.), or propafenone (0.01, 0.03, 0.1, and 0.3 mg/kg i.v.) to dogs with sustained AF resulted in termination of AF in five of six, four of seven, four of six, and five of six animals tested, respectively (representative studies, Fig. 6). Hence, all test agents seemed comparably effective in terminating inducible AF in these relatively small groups. All test agents terminated AF in one half or more of animals tested. Conversely, none of the test agents terminated AF in all animals tested. Each of the seven animals entered into the study had sustained AF terminated by at least one of the test agents. In this small cohort of responding animals, there was no apparent association in efficacy between individual agents, i.e., successful AF termination with one agent did not predict efficacy with any other agent.

Efficacious doses of test agents terminating AF varied among animals and studies, with ranges as follows: MK-499,
Fig. 6. Termination of sustained AF in conscious dogs with underlying heart failure. In each example, top panel is lead II ECG, and bottom panel is atrial electrogram. A, termination of AF at 4 min 24 s following i.v. administration of 0.001 mg/kg i.v. MK-499; B, termination of AF at 6 min 30 s following i.v. administration of 1.0 mg/kg i.v. ISQ-1. C, termination of AF at 3 min 25 s following i.v. administration of 0.03 mg/kg i.v. TAEA. D, termination of AF at 5 min following i.v. administration of 0.3 mg/kg i.v. propafenone.

0.001 to 0.01 mg/kg (0.003 ± 0.002 mg/kg); ISQ-1, 0.03 to 1.0 mg/kg (0.34 ± 0.23 mg/kg); TAEA, 0.03 to 0.3 mg/kg (0.17 ± 0.08 mg/kg); and propafenone, 0.03 to 0.3 mg/kg (0.25 ± 0.05 mg/kg). Time to termination of AF following the administration of individual doses of test agents also varied from study to study from as short as 40 s following i.v. bolus dosing to as long as approximately 10 min following high dose. Accordingly, plasma levels of test agents determined at the time of AF termination varied among animals and studies, with ranges of as follows: MK-499, 10.6 to 107.0 nM (32.6 ± 18.7 nM); ISQ-1, 88 to 1309 nM (817 ± 274 nM); TAEA, 58 to 1957 nM (714 ± 622 nM); and propafenone, 105 to 1592 nM (816 ± 240 nM).

Figure 7 summarizes changes from baseline, pre-AF induction, to 2 min post-AF termination in ARP, VRP, QTc, and heart rate (HR) in those animals responding to test agents with AF termination. In this relatively small cohort of responding animals, the only cardiac electrophysiological effect achieving statistical significance was an increase in VRP with MK-499. There were no significant changes in VRP with ISQ-1, TAEA, and propafenone, and no apparent changes in QTc with any of the test agents. Heart rate was reduced significantly by propafenone at 2 min post-AF termination. MK-499 also tended to reduce heart rate, although more variably, at 2 min post-AF termination. Changes in heart rate with ISQ-1 and TAEA were minor and variable.

-directed Acute Cardiac Electrophysiologic Studies in Dogs Using Intravenous Bolus (Simulating AF Termination Study Administration) Plus Infusion Dosing.

Directed cardiac EP studies were conducted in anesthetized dogs using i.v. bolus dosing regimens of TAEA and ISQ-1 (simulating administration of these test agents in AF termination studies) plus continuous i.v. infusions of these test agents (simulating the initial acute cardiac EP studies). Figures 8 and 9 summarize the cardiac EP effects of TAEA and ISQ-1, respectively, using these i.v. bolus plus infusion regimens. Both TAEA and ISQ-1 elicited significant maximal 12 to 15% increases in ARP, comparable significant increases in A-H interval, and more modest (5–7%) but consistent and significant increases in P-A interval compared with vehicle (Figs. 8A and 9A). Neither TAEA nor ISQ-1 significantly altered VRP, H-V, and HEG intervals compared with vehicle (Figs. 8B and 9B). Plasma concentrations of TAEA achieved at approximately 2 min following bolus i.v. administrations of 0.01, 0.03, 0.1, and 0.3 mg/kg (with ongoing 0.7 μg/kg/min infusion) were 25.3 ± 6.1, 71.0 ± 23.7, 172.3 ± 29.2, and 494.0 ± 94.6 nM, respectively. Plasma concentrations of ISQ-1 achieved at approximately 2 min following bolus i.v. administrations of 0.03, 0.1, 0.3, and 1.0 mg/kg (with ongoing 7.0 μg/kg/min infusion) were 423.3 ± 111.3, 799.3 ± 115.4, 1559.0 ± 207.8, and 4115.0 ± 677.9 nM, respectively.

Discussion

Animal models in which AF occurs in the setting of underlying cardiac pathology include rapid atrial pacing to induce atrial electrical remodeling (Morillo et al., 1995; Gaspo et al., 1997), mitral regurgitation inducing atrial histological remodeling (Everett et al., 2000), acute atrial ischemia (Sinno et al., 2003), and rapid ventricular pacing resulting in heart failure. The canine model in which rapid ventricular pacing (generally 2–6-week duration) produces ventricular dysfunc-

tion, leading to atrial remodeling and the ability to sustain AF, has been characterized extensively in the literature. Rapid ventricular pacing has been reported to increase atrial dimension (Shi et al., 2001), pressure (Cha et al., 2004), and fibrosis (Li et al., 1999, 2001; Cha et al., 2004; Hanna et al., 2004). Biochemically, increased atrial angiotensin II concentration, increased Bax/Bcl-2 ratio, increased expression of phosphorylated mitogen-activated protein kinases, and tran-
siently increased apoptosis have been reported previously (Li et al., 2001; Cardin et al., 2003; Hanna et al., 2004). Increased heterogeneity of conduction and areas of slow conduction in atria, accompanied by decreases in transient outward current, T-type calcium current, and slowly activating component of delayed rectifier potassium current, but no change in inward rectifier potassium current, $I_{Kr}$, and $I_{K1}$ have been reported in electrophysiological studies (Li et al., 1999, 2000b; Cha et al., 2004). Both reentrant and focal mechanisms underlying AF have been reported in atrial mapping studies (Li et al., 2000a; Derakhchan et al., 2001; Ryu et al., 2005; Everett et al., 2006).

Pharmacologically, chronic treatment with the ACE inhibitor enalapril attenuated biochemical alterations, reduced fibrosis, and decreased the duration of AF in this model (Li et al., 2001; Cardin et al., 2003). The acute administration of the $I_{Kr}$ blocker dofetilide terminated ongoing AF and prevented the induction of sustained AF in this model (Li et al., 2000a).

The principle finding of the present studies was the demonstration of termination of sustained AF with two structurally distinct Kv1.5 blockers ISQ-1 and TAEA in the conscious canine heart failure model. Compounds that block Kv1.5 as part of their spectra of pharmacological actions have been...
shown previously to terminate atrial arrhythmias in other animal models: NIP-142, DPO-1, and ISQ-1 in dog acute atrial surgical or crush injury flutter models (Nagasawa et al., 2002; Stump et al., 2005; Regan et al., 2007); RSD1235 and NIP-142 in dog acute vagotonic AF models (Nattel et al., 2001; Nagasawa et al., 2002), AVE-0118 in a pig model of acute vulnerability to atrial tachycardia induction (Knobloch et al., 2002), and AVE-0118 and RSD1235 in dog and goat models of rapid atrial pacing-induced atrial electrical remodeling and AF (Beatch et al., 2002, 2004; Blaauw et al., 2004). The present observation of AF termination with the Kv1.5 blockers ISQ-1 and TAEA in the dog heart failure model extends the profile of atrial antiarrhythmic efficacy of Kv1.5 blockade.

Interestingly, threshold doses of the Kv1.5 blockers terminating AF in the conscious heart failure dogs were not accompanied by measurable increases in ARP determined at 2 min post-AF termination. In contrast, in the initial acute cardiac EP assessments conducted in normal chloralose-anesthetized dogs, continuous i.v. infusions of ISQ-1 and TAEA elicited increases in atrial refractoriness with no change in ventricular refractoriness at pacing rate tested or ECG intervals, including QTc, consistent with an atrial-selective action. Similarly, in directed acute cardiac EP studies in dogs using i.v. bolus dosing regimens (consistent with the AF termination studies) plus continuous i.v. infusions (consistent with the initial acute cardiac EP studies, designed to maintain plasma test agent levels), ISQ-1 and TAEA elicited increases in atrial refractoriness and conduction times with no change in ventricular refractoriness and conduction at pacing rate tested. It is uncertain whether or to what extent increases in atrial conduction times observed with ISQ-1 and TAEA might be secondary to effects on atrial refractoriness. Of note, atrial versus ventricular selectivities of effect were maintained for TAEA and ISQ-1 over the wide range of plasma levels achieved in the initial cardiac EP continuous i.v. infusion profile (58.5 ± 23 and 330.3 ± 43.5 nM at end infusions, respectively) versus the directed i.v. bolus plus infusion cardiac EP study (494.0 ± 94.6 and 4115.0 ± 677.9 nM following high doses, respectively). Also of note, increases in atrial refractoriness were observed with continuous i.v. infusion TAEA at plasma levels approximately one fourth of the IC50 for block of Kv1.5 in Chinese hamster ovary cells expressing human Kv1.5 (238 nM). This apparent difference in activity/potency measures may reflect tissue accumulation of TAEA in vivo, species differences in the Kv1.5 channels in the two test systems, or general differences between in vitro and in vivo assays.

Potential reasons for the apparent lack of effect of the Kv1.5 blockers on ARP acutely post-AF termination are manyfold. Termination of AF following bolus i.v. dosing in conscious dogs may have resulted from threshold modulation of critical reentrant circuits or focal triggers, which may not have been reflected in the measurement of ARP at one fixed site or pacing rate. Conversely, the continuous i.v. infusion regimens used in the EP studies in anesthetized normal dogs versus bolus dosing in the heart failure AF dogs may have allowed for more stable plasma test agent levels and effects, promoted tissue accumulation of test agent, and facilitated the generalized measurement of effect on ARP. Potential frequency dependence of effects of the Kv1.5 blockers during AF versus in the post-AF termination setting also may have influenced the observed effects. Although the magnitude of IKur in human atrial myocytes has been shown to be inherently reverse frequency-dependent (Feng et al., 1998), block of IKur has been reported to be frequency-independent with NIP-141/142 and RSD1235 (Matsuda et al., 2001; Fedida et al., 2005) and forward frequency-dependent with AVE-0118 and DPO-1 (Decher et al., 2006; Lagrutta et al., 2006). It also should be remembered that the apparent lack of effect of test agents on ARP acutely post-AF termination must be viewed in the context that even short periods of AF significantly shorten atrial refractoriness both in dog (Miyata et al., 2002) and in human (Daoud et al., 1996). Finally, it cannot be precluded that effects of anesthesia in normal dogs versus conscious studies in heart failure AF dogs may have influenced measurement of effect on ARP.

Termination of AF in the present dog heart failure AF preparation with the prototype IKr blocker MK-499 is consistent with the previous demonstration of AF termination efficacy with dofetilide in a similar model (Li et al., 2000a), and the clinical efficacy of dofetilide in AF termination. The prolongation of ventricular refractoriness with MK-499 in the heart failure AF dogs is consistent with block of ventricular IKr. Termination of AF with the class IC sodium channel blocker propafenone in the present studies also is consistent with clinical efficacy for AF termination with this agent. Plasma concentrations of propafenone determined post-AF termination in the present studies (mean 0.8 μM) were approximately 4-fold lower than those reported clinically for acute i.v. AF termination (Kingma and Suttorp, 1992).

None of the test agents assessed in the present study were universally effective in terminating AF in all animals tested. This probably reflects heterogeneity in the anatomic substrates for AF in the animals entered into study. Variation across test agent studies in the dose required for AF termination and time postdose for termination also likely reflects heterogeneity in substrate from animal to animal, and possibly test day to test day. Accordingly, variations in test agent dose required for AF termination, time to termination postdose, and the use in the AF termination studies of bolus i.v. test agent dosing, which is inherently prone to peak-trough swings in plasma concentration, all likely contributed to variation in plasma concentrations determined post-AF termination. It is possible that the use of more prolonged continuous i.v. infusion dosing or chronic oral dosing might have reduced variability in dose or plasma concentration associated with AF termination efficacy in this model.

The major caveat of the present study, and a caveat common to all animal studies, is uncertainty regarding relevance and relative roles of Kv1.5 and IKur in dog versus human. Kv1.5 and IKur, are known to be important in human (Feng et al., 1998) and canine (Fedida et al., 2003) atria. However, the predictiveness of dog, and in particular this canine heart failure AF model, to antiarrhythmic efficacy in human for the targeting of this channel is unknown. Ultimately, a thorough clinical assessment of a selective Kv1.5 blocker would be required to ascertain the predictiveness of this animal model and therapeutic utility of this proposed antiarrhythmic mechanism of action.
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References


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