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MK-0448, a Specific Kv1.5 Inhibitor: Safety, Pharmacokinetics and Pharmacodynamic Electrophysiology in Experimental Animal Models and in Humans.

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MK-0448, a Specific Kv1.5 Inhibitor: Safety, Pharmacokinetics and Pharmacodynamic Electrophysiology in Experimental Animal Models and in Humans

Running title: *Pavri et al.; Preclinical/ FIH studies of a new Kv1.5 inhibitor*

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Abstract

Background -We evaluated the viability of I_{Kur} as a target for maintenance of sinus rhythm in patients with a history of atrial fibrillation through the testing of MK-0448, a novel I_{Kur} inhibitor.
Methods and Results - *In vitro* MK-0448 studies demonstrated strong inhibition of I_{Kur} with minimal off-target activity. *In vivo* MK-0448 studies in normal anesthetized dogs demonstrated significant prolongation of the atrial refractory period compared with vehicle controls without affecting the ventricular refractory period. In studies of a conscious dog heart failure model, sustained AF was terminated with bolus intravenous MK-0448 doses of 0.03 and 0.1 mg/kg. These data led to a two-part "first-in-human" study: Part I evaluated safety and pharmacokinetics, and Part II was an invasive electrophysiologic (EP) study in healthy subjects. MK-0448 was well-tolerated with mild adverse experiences, most commonly irritation at the injection site. During the EP study, ascending doses of MK-0448 were administered, but no increases in atrial or ventricular refractoriness were detected despite achieving plasma concentrations in excess of 2 μM. Follow-up studies in normal anesthetized dogs designed to assess the influence of autonomic tone demonstrated that prolongation of atrial refractoriness with MK-0448 was markedly attenuated in the presence of vagal nerve simulation, suggesting that the effects of I_{Kur} blockade on atrial repolarization may be negated by enhanced parasympathetic neural tone.
Conclusions - The contribution of I_{Kur} to human atrial electrophysiology is less prominent than in preclinical models and therefore is likely to be of limited therapeutic value for the prevention of atrial fibrillation.

Key words: arrhythmia; atrial fibrillation; electrophysiology; pharmacokinetics

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Atrial fibrillation, the most common sustained cardiac arrhythmia, is associated with considerable morbidity as well as mortality.[1-3] This condition may be effectively managed by either rhythm control (cardioversion) or rate control, depending on the patient profile. For patients who are appropriate candidates for cardioversion, there are several strategies for conversion of atrial fibrillation to sinus rhythm, including electrical or pharmacologic methods.[4]

Currently available pharmacotherapies include flecainide, propafenone, amiodarone, and ibutilide, which are sodium channel blockers that block sodium ion channel proteins such as $Na_v1.5$ and/or potassium channel blockers that block the rapidly activating delayed rectifier cardiac potassium current (I_{Kr}) and/or the slowly activating delayed rectifier cardiac potassium current (I_{Ks}).[5,6] While these agents have modest efficacy in restoring sinus rhythm, they also have unwanted effects on ventricular depolarization (QRS widening due to slowed conduction) and repolarization (QT interval prolongation), which may lead to ventricular arrhythmias such as ventricular tachycardia or torsade de pointes, particularly in patients with structural heart disease.[4,7,8]

Due to the risk of these serious adverse effects, a more targeted approach to therapy is an important goal.[8] I_{Kur} is an ultra-rapid delayed rectifier current that is different from other currents such as I_{Kr} due to its presence in atrial myocytes but absence in ventricular tissue.[9] Blockade of I_{Kur} has been shown to selectively prolong atrial refractoriness without affecting ventricular repolarization and appears to be an effective antiarrhythmic strategy for the acute conversion of atrial fibrillation in animal models.[10] For these reasons, I_{Kur} blockade may represent an atrial-specific strategy that can lead to therapies that are effective in converting atrial fibrillation yet do not have ventricular effects. However, research into this target has led to contradictory evidence, suggesting that suppression of I_{Kur} in certain circumstances may promote rather than prevent

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atrial fibrillation. A loss-of-function mutation in KCNA5, the gene that encodes Kv1.5 channels responsible for I_{Kur}, has been described in a familial case of atrial fibrillation.[11] In isolated human atrial myocytes, exposure to 4-aminopyridine at concentrations reported to selectively block I_{Kur} resulted in action potential prolongation, a propensity to elicit early after-depolarizations and triggered activity with co-exposure to isoproterenol.[11] Similarly in normal mice, the intraperitoneal administration of 4-aminopyridine provoked premature atrial complexes, with atrial fibrillation induced by co-administration of isoproterenol.[11] Further, experimental evidence has shown that exposure to 4-aminopyridine allowed induction of atrial fibrillation in healthy isolated canine coronary-perfused right atrial preparations and failed to terminate fibrillation in remodeled atrial preparations.[12] Data from Wettwer et al suggest that whether inhibition of I_{Kur} prolongs or shortens action potential duration (APD) depends on the disease status of the atria and is determined by the level of electrical remodeling.[13] They concluded that I_{Kur} blockade shortens action potential duration at 90% (APD_{90}) in sinus rhythm, but prolongs it in AF. This is probably due to the observation that normal (healthy) atria typically displayed action potentials with a prominent plateau, whereas remodeled atria displayed triangular-shaped APs.[12]

We developed MK-0448 (*N*-{6-[(1S)-1-(4-fluorophenyl)-2,2-di(pyridin-3-yl)ethyl]pyridin-2-yl}methanesulfonamide) in order to test the hypothesis that specific blockade of I_{Kur} would be an effective pharmacologic approach for the termination and/or prevention of atrial fibrillation without the ventricular effects that are associated with other anti-arrhythmics. MK-0448 is an agent that is structurally related to triarylethanolamine, 2-phenyl-1,1-dipyridin-3-yl-2-pyrrolidin-1-yl-ethanol (TAEA), a probe compound demonstrated to have atrial specificity and anti-arrhythmic activity in an animal model of atrial fibrillation.[10] TAEA was the basis for

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MK-0448, which was generated to optimize various pharmacokinetic properties while maintaining specificity for Kv1.5, a potassium channel that is responsible for $I_{Kur.}$[14] Additionally, isoquinolinone (ISQ-1), a structurally distinct compound that also targets Kv1.5, was also previously shown to be significantly more effective at terminating atrial fibrillation than placebo in a dog heart failure AF model.[10] We conducted *in vitro* and *in vivo* studies, as well as first-in-human studies with electrophysiologic testing using MK-0448 to examine whether blockade of Kv1.5 (and hence I_{Kur}) would increase atrial refractoriness and thereby demonstrate potential as a treatment for atrial fibrillation in humans.

Methods

Preclinical Program

Detailed methodologies for preclinical *in vitro* studies are provided in Appendix 1. Presented here is a summary of methods for the *in vitro* and *in vivo* studies.

In vitro Studies

Inhibition of currents carried by heterologously expressed Kv1.5 and native I_{Kur} channels was assessed using Chinese hamster ovary (CHO) cells and human atrial myocytes, respectively, in functional voltage-clamp assays with traditional patch-clamp electrophysiological methods to determine potency. Ventricular repolarization was studied through testing effects on hERG channels heterologously expressed in CHO-K1 cells using standard whole-cell voltage clamp techniques. Current amplitudes recorded pre-drug and post-drug were compared pair-wise to determine inhibitory effect. Percent inhibition of the peak control current was plotted as a function of compound concentration and the concentration of drug required to inhibit current by 50 % (IC_{50}) was determined by fitting of the Hill equation to the concentration response data: % of Control = $100 \bullet (1 + ([Drug]/IC_{50})^{p})^{-1}$. We also tested effects on slowly activating cardiac

delayed rectifier K^+ current, I_{Ks}, resulting from the stable co-expression of the ion channel subunits hKCNQ1 and hKCNE1 in HEK-293 host cell line. Specific assays for I_{K1}, I_{to}, I_{Na}, Kv3.2, IK_{Ca}, Kv2.1, Kv1.7, and NR1a/2B were also performed.

In vivo Studies

Cardiac Electrophysiological Studies in Anesthetized Dogs

The electrocardiographic and cardiac electrophysiological effects of MK-0448 were compared with vehicle-matched control infusions in anesthetized male or female mongrel dogs (6.3-12.1 kg). The surgical preparation and methods for the measurement of cardiac electrophysiologic parameters in anesthetized dogs were as described previously.[15] Dogs were anesthetized with α-chloralose (100 mg/kg i.v.) and minimal sodium pentobarbital (5 mg/kg i.v.) to provide additional analgesia and anesthesia. A continuous infusion of α-chloralose (10 mg/kg/hr i.v.) was utilized to maintain anesthesia over the time course of the study.[15] At 30 min following surgical preparation, the following parameters were measured prior to (baseline) and at 15, 30, 45 and 60 min following initiation of 60 min continuous IV infusions of vehicle or test agent: sinus heart rate, mean arterial pressure, ECG intervals including rate-corrected QT interval [(QT in msec)/√(R-R in sec)], atrial and ventricular excitation thresholds (AET, VET), and atrial and ventricular relative refractory periods (ARRP, VRRP). ARRP and VRRP are commonly used measures of refractory period in intact animal cardiac electrophysiology studies (10, 15); in this study, they were determined using programmed pacing at a basic S1-S1 cycle length of 400 msec (150 bpm), introducing S2 at 2xET and decrementing the S1-S2 initially at 10msec intervals followed by 2msec intervals until an S2 extrastimulus could not be propagated.. Blood samples for determination of plasma concentrations of MK-0448 by liquid chromatography - tandem mass spectrometry (LC/MS/MS) were also obtained at these time points.

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Two temporally distinct sets of *in vivo* cardiac electrophysiological studies were conducted. Lower dose range studies were conducted in order to determine the pharmacologically effective dose/plasma concentration of MK-0448, and consisted of four groups of dogs assigned to i.v. treatment with vehicle (25% hydroxypropyl-ß-cyclodextrin aqueous; n=6) or MK-0448 at 0.15 (n=4), 0.30 (n=5) or 0.45 µg/kg/min (n=5) infused at a rate of 20 ml/hr. Subsequently, higher dose range studies were conducted, consisting of three groups of dogs assigned to i.v. treatment with vehicle (25% hydroxypropyl-ß-cyclodextrin aqueous; n=2) or MK-0448 at 20.0 (n=4) or 30.0 µg/kg/min (n=4) infused at a rate of 20 ml/hr.

Electrophysiologic Study of Atrial Fibrillation in a Dog Heart Failure Model

The surgical preparation and methods for the production of heart failure by rapid right ventricular pacing as well as cardiac electrophysiologic testing and induction of atrial fibrillation (AF) by atrial burst pacing are previously described.[10] In these 3 animals, MK-0448 dosing was initiated after 15 minutes of sustained AF. MK-0448 was administered as 30 sec intravenous infusions at 5 min intervals at sequential doses of 0.03, 0.1, 0.3 and 1.0 mg/kg. Blood samples were drawn prior to dosing, at termination of AF or at 5 minutes after the highest dose tested if AF was not terminated.

First in Human (FIH) Study

The first in human study (Sponsor Protocol 001) was conducted from August 2005 to February 2006 following approval by the Thomas Jefferson University (TJU) Investigational Review Board and the TJU Hospital and Clinical Pharmacology Unit (CPU) and was conducted in accordance with Good Clinical Practice principles. Prior to enrollment, all patients provided written informed consent.

This was a two-part study. Part I was done to evaluate the safety and tolerability of

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intravenous (IV) doses of MK-0448, obtain pharmacokinetic parameters, and to evaluate urinary excretion and pharmacodynamic effects on heart rate, blood pressure, and ECG parameters. Part II used novel techniques to evaluate the electrophysiological effects of MK-0448 on atrial and ventricular refractory period duration, as well as sinus node, AV node, and His-Purkinje function.

Part I was a double-blind, randomized, placebo-controlled, multiple-period, alternating panel, rising-dose study in healthy male subjects. Two panels (Panels A and B) consisting of 8 subjects each alternatively received single IV rising infusions of MK-0448 or matching placebo in a 3:1 ratio, respectively, in up to 5 treatment periods. Doses for Panel A were 0.5 mg, 2 mg, 8 mg, 18 mg, and 32 mg for each successive period. Doses for Panel B were 1 mg, 4 mg, 12 mg, 24 mg, and 40 mg for each successive period. At least 3 days elapsed before administration of the next higher dose. All doses were administered for 2 hours (sterile solution for IV 0.1 mg/ml in a 50 mL vial).

An additional serial panel (Panel C) was conducted following a satisfactory evaluation of safety, tolerability, and preliminary pharmacokinetic data from all doses administered in Panels A and B. Panel C (8 subjects; 6 active, 2 placebo), was administered as single IV infusions for 2 hours in a 5-period single ascending-dose design with doses at 40 mg, 50 mg, 62 mg, 80 mg, and 100 mg for each successive period.

Safety measures included telemetry monitoring, 12-lead ECGs, physical examinations, and vital signs including measurement of orthostatic heart rate and blood pressure. During and after infusions of MK-0448, specimens were obtained for pharmacokinetic analysis.

Part II was a placebo-controlled, rising, single-dose study in healthy males (Panel D). Doses in Part II (panel D) were about half of the starting doses in Panels A and B in Part 1.

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Electrophysiology studies were performed after overnight fasting. Caffeinated beverages and tobacco use were not allowed during the study period. The starting dose for Part II was selected to target a C_{eoi} (concentration at the end of infusion) of 100 nM based on the pharmacokinetic data from Part I and was expected to be ~2 mg delivered over 2 hours, based on allometric scaling of pharmacokinetic data from preclinical experiments. Each subject had 3 venous sheaths placed percutaneously in the femoral veins after infiltration of lidocaine without epinephrine. None of the subjects received systemic anesthetics or sedation during the procedure. Through those sheaths, quadripolar catheters were advanced into the heart under fluoroscopic guidance using the standard procedures and techniques that have been established for clinical electrophysiology studies. Blood pressure was monitored every 10 minutes with an inflatable cuff on the upper arm. The 3 catheters were placed in the right atrium, in the region of the His bundle, and in the right ventricle. Placement was confirmed fluoroscopically and with continuous monitoring of the intracardiac electrograms. After a 15-minute baseline period, sinus node recovery times, atrial effective refractory period (AERP), AV node refractoriness and Wenckebach cycle length, His-Purkinje reserve, and ventricular effective refractory period (VERP) were measured. Standard protocols for programmed electrical stimulation were used. Pacing was performed at twice diastolic threshold. Atrial ERP measurements were performed from the high right atrial location at two drive cycle lengths: 600 ms and 400 ms. Premature impulses were delivered after a drive train of 8 impulses. The degree of prematurity was increased (the coupling interval was shortened) in 10 ms decrements until failure to capture was noted on two consecutive runs. Ventricular programmed stimulation was performed in the exact same fashion from the RV apex.

After the initiation of the IV infusion of MK-0448 or placebo, sets of atrial and

ventricular effective refractory period measurements were made every 20 minutes. After each set of effective refractory period measurements, PK samples were obtained from the side ports of the femoral venous sheaths. At the end of the 2-hour infusion, measurements of sinus node recovery times, AERP, AV node refractoriness and Wenckebach cycle length, His-Purkinje reserve, and VERP were repeated. In the hour following the discontinuation of the infusion, additional sets of AERP and VERP measurements were made every 20 minutes along with PK sampling. The catheters were then removed, the venous sheaths were removed, hemostasis was attained by manual compression, and the subjects were transported back to the Clinical Research Unit (CRU) where they were monitored overnight.

Confirmatory In Vivo Studies in Dogs: Atrial Refractoriness in Anesthetized Dogs with Vagal Nerve Stimulation

A follow-up study in anesthetized dogs was specifically designed to assess the influence of autonomic tone on the effects of MK-0448 on atrial refractoriness. In order to control autonomic status in the present study, the α-chloralose/pentobarbital anesthetic regimen used in the previous cardiac electrophysiologic study in dogs was replaced with an α-chloralose/morphine regimen to preclude effects of pentobarbital on vagal tone, and nadolol was administered to control sympathetic tone. Mongrel dogs (male or female, 6.7-12.0 kg) were anesthetized with α-chloralose (100 mg/kg i.v.) and morphine (2 mg/kg i.m.), and ventilated with room air via an endotracheal tube. The left and right femoral veins were cannulated for blood sampling and for treatment infusion, and the left femoral artery was cannulated for blood pressure monitoring. Pin electrodes were attached for the monitoring of Lead II ECG. Both cervical vagal nerve trunks were isolated and ligated rostrally. Bipolar hook electrodes composed of 0.25-mm-diameter Teflon-coated silver wire, with insulation removed at the terminal 1-2 mm, were inserted into the

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middle of each vagal nerve by means of 18 gauge hypodermic needles. The vagal nerve electrodes were advanced caudally within and parallel to vagal fibers for several centimeters. A left thoracotomy was performed at the 4th intercostal space, and the heart was exposed following pericardial incision and cradled. A stainless steel epicardial bipolar electrode was sutured to the right atrium for pacing, and a stainless steel epicardial quadripolar electrode was sutured to the left atrium for sensing left atrial electrograms and for the determination of AET and ARRP (2 msec pulse duration, 2x AET, 150 bpm pacing rate). Following surgical preparation, a bolus dose of 0.5 mg/kg nadolol i.v. was administered, and the preparation was allowed to equilibrate for 20 min.

At 30 min following surgical preparation, animals were randomized to i.v. treatment infusion with either vehicle alone (n=4, 25% aqueous hydroxypropyl-ß-cyclodextrin, 10 ml infused over 30 min) or 1.0 μg/kg/min MK-0448 administered in identical vehicle and infusion rate (n=4, in vehicle of 25% aqueous hydroxypropyl-ß-cyclodextrin, 10 ml infused over 30 min). Based on previous studies with MK-0448 in chloralose/pentobarbital-anesthetized dogs, the 1.0 μg/kg/min i.v. infusion was expected to elicit a robust increase in atrial refractory period in the absence of alteration in autonomic tone. The following measurements were obtained at baseline (pretreatment) and at 15 and 30 min of treatment infusion: heart rate, Lead II ECG intervals including QTc using Bazett's formula, AET and ARRP. At each time point in both groups, measurements were obtained sequentially during bilateral vagal stimulation (5.0 volts, 0.1 msec pulse duration) at vagal nerve stimulation frequencies of 0 Hz (i.e. no vagal pacing), 2 Hz and 5 Hz. In the MK-0448 treatment groups, blood samples were drawn at baseline (pretreatment) and at 15 and 30 min of infusion for the determination of plasma [MK-0448].

Changes in ARRP are expressed as percentage changes from baseline within indicated

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vagal nerve stimulation frequency.

Results

Specificity of MK-0448 for Kv1.5 channels and its associated current, I_{Kur}

MK-0448 targeted Kv1.5, the major pore-forming subunit underlying native I_{Kur}, with an IC_{50} of 8.6 nM for recombinant human Kv1.5 (hKv1.5) heterologously expressed in CHO cells (Figure 1). Similarly, MK-0448 potently inhibited I_{Kur} in human atrial myocytes with an IC_{50} of 10.8 nM. MK-0448 also demonstrated a potent effect on currents associated with other Kv1.x and Kv2.x subunits, such as Kv1.7 and Kv2.1, with voltage clamp-derived IC_{50} values of 72 nM and 61 nM, respectively (Table 1).

While MK-0448 strongly blocked I_{Kur}, its effect on other cardiac currents was much weaker, indicating a high degree of selectivity for I_{Kur}. Compared with the low-nM range for I_{Kur}, MK-0448 IC_{50} values were in the μM-range for other cardiac currents including I_{Kr} (heterologously expressed hERG; voltage-clamp IC_{50} = 110 μM), I_{TO} (heterologously expressed Kv4.3; voltage-clamp IC_{50} = 2.3 μM), I_{Na} (heterologously expressed SCN5a; inactive up to 10 μM), Kv3.2 (voltage-clamp IC_{50} = 6.1 μM), and IK_{Ca} (voltage-clamp IC_{50} = 10.2μM). Although MK-0448 was generally highly selective against currents expressed in the cardiac ventricles, it did exhibit moderate inhibition of the slowly activating cardiac delayed rectifier K+ current, I_{Ks}, with an IC_{50} of 0.79 μM (determined by voltage clamp experiments in HEK-293 cells with stable co-expression of the ion channel subunits hKCNQ1 and hKCNE1). Activity against I_{Ks}, however, was seen at a 70-fold higher concentration of MK-0448 than inhibition of I_{Kur}.

Selective Effect of MK-0448 on ARRP in Anesthetized Dogs

Since MK-0448 demonstrated selectivity for I_{Kur} versus ventricular ion channel currents, we investigated whether atrial repolarization might be prolonged preferentially to ventricular

repolarization in 3 anesthetized mongrel dogs (two male and one female, 20-26 kg). The effects of MK-0448 were assessed in comparison to vehicle-matched control infusions. Continuous i.v. infusions of MK-0448 at doses of 0.30 and 0.45 μg/kg/min resulted in exposure-dependent increases in atrial refractory period (ARRP) without changes in ventricular refractory period (VRRP); heart rate; mean arterial pressure; or ECG PR, QRS, and QTc intervals. At a plasma concentration of approximately 36 nM, MK-0448 increased ARRP by 10%. In contrast, a mean plasma concentration of ~6 μM was required to produce changes from baseline of ~5% in VRRP and QTc. Increases in mean arterial pressure ~15-20 mmHg compared with controls were also noted at the elevated plasma concentration of ~6 μM.

Figure 2 shows an integrated PK-PD plot of exposure versus change in ARRP and VRRP from experiments in anesthetized dogs. The vertical dashed lines indicate the magnitude of the window between the exposures that selectively affect the atrium versus exposures that affect both atrium and ventricle. These data indicate that at exposures of MK-0448 that are associated with up to an approximate 20% prolongation of ARRP, there is no appreciable effect on ventricular repolarization. These data further suggest that effects on ventricular repolarization are seen at exposures of MK-0448 that are approximately 100-fold above those that prolong atrial repolarization.

Effect of MK-0448 on Atrial Fibrillation in a Dog Heart Failure Model

To directly investigate the antiarrhythmic effects of MK-0448 in the dog model, atrial fibrillation was induced by atrial burst pacing in dogs with heart failure produced by rapid, sustained right ventricular pacing. The cardiac hemodynamic status of these rapid ventricular pacing-induced heart failure dogs has been described previously, and was characterized by an increase in left ventricular end diastolic pressure, and decreases in left ventricular systolic pressure and +dP/dt.[10]

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Sustained AF in this model was terminated in two of three animals tested with intravenous doses of 0.03 mg/kg i.v. (termination at 30 sec after administration) and 0.1 mg/kg i.v. MK-0448 (termination at 90 sec after administration), yielding plasma concentrations of 1.7 and 5.9 μM respectively at the times of AF termination (Figure 3). In the third animal, AF failed to terminate after the administration of sequential intravenous doses up to 1.0 mg/kg MK-448 on two separate study dates, yielding plasma concentrations of 16.9-18.7 μM at 5 minutes after the final dose. Furthermore, in that series none of the seven vehicle-treated controls converted to sinus rhythm.[10] These data suggest that concentrations of MK-0448 that yield significant ARRP prolongation and no or minimal lengthening of VRRP in the dog may be suitable for acute conversion of atrial fibrillation.

Safety, Tolerability and Pharmacokinetic findings of MK-0448 in Healthy Young Human Subjects

Given the selectivity of MK-0448 for atrial repolarization and its anti-arrhythmic effects in the dog model, MK-0448 was tested in Phase I studies for its electrophysiologic effects in humans. There were 24 healthy, male subjects (age range 23-44 years) who participated in Part 1 of the trial. In part 2, there were 6 subjects enrolled. Four subjects discontinued the trial; 1 subject discontinued due to a benign accelerated idioventricular rhythm, 1 subject withdrew for personal reasons, and another subject was lost to follow up. An additional subject discontinued Part II due to an adverse experience of atrial fibrillation that occurred during baseline programmed electrical stimulation, prior to dosing.

Supplemental Tables 1 and 2 (Appendix 2) show adverse experiences for Part 1 and 2, respectively. There were no serious adverse experiences and no discontinuations due to adverse experiences. All adverse experiences were transient and mild in intensity. The most common

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adverse experience was irritation at the site of injection. Mean $AUC_{0-\infty}$ and concentration at the end of the infusion (C_{eoi}) appear to increase proportionally with dose through 100 mg to maximal values of 22904 nM·hr and 5726 nM, respectively (Supplemental Table 3; Appendix 2).

Electrophysiologic Effects of MK-0448 in Healthy Young Human Subjects

An invasive electrophysiologic assessment was incorporated into Part II of the study to directly measure the effect of MK-0448 on atrial and ventricular refractory periods. In Part II, 5 of the 6 enrolled subjects completed dosing; 1 subject did not dose because of the onset of an AE that occurred before dosing that was unrelated to study medication. The 5 completed subjects were administered ascending doses of up to 32 mg of MK-0448 over a 2-hour infusion. In contrast to expectations, there were no increases in AERP or VERP at any dose of MK-0448 (Table 2; Figure 4) despite pharmacokinetic analysis confirming plasma concentrations of up to ~2.1 μM at the highest administered dose. Results for individual patients in the study are in Supplement Figure 1. Thus, despite preclinical data suggesting that MK-0448 preferentially prolongs atrial repolarization in a heart failure dog model, there was no evidence that MK-0448 influenced atrial (or ventricular) repolarization in healthy human volunteers at concentrations up to 2 μM (corresponding to > 50-fold higher than the concentration at which significant AERP prolongation was observed in the dog).

Interaction of MK-0448 and Vagal Nerve Stimulation on Atrial Repolarization in Anesthetized Dogs

Because the human subjects tested in Phase I were healthy, young, and presumably had high vagal tone, and that heart failure is known to be associated with vagal withdrawal, we hypothesized that vagal stimulation might influence the effect of I_{Kur} blockade on prolongation of atrial repolarization. Accordingly, a follow-up study in anesthetized dogs was specifically

designed to assess the influence of autonomic tone, and in particular vagal nerve stimulation, on the effects of MK-0448 on atrial refractoriness. As expected, enhanced parasympathetic neural tone produced by bilateral vagal nerve stimulation in the dog model resulted in vagal stimulation-dependent decreases in heart rate and ARRP. In the absence of vagal nerve stimulation, 1.0 μg/kg/min i.v. MK-0448 produced robust (approximately 20 msec, 20%) increases in ARRP at both 15 and 30 min of infusion. In contrast, increases in ARRP in the MK-0448 group during both 2 and 5 Hz vagal nerve stimulation were diminished markedly as compared with the absence of vagal stimulation, such that during 2 Hz and 5 Hz vagal nerve stimulation, increases in ARRP were comparable in the MK-0448 and vehicle treatment groups. Figure 5 compares percent changes in ARRP from baseline in the vehicle and MK-0448 treatment groups, respectively, at 30 min of treatment infusion. In the vehicle-treatment group, only slight increases in ARRP were observed over the duration of the study both in the absence and presence of vagal nerve stimulation. Overall, these data suggest that the effect of I_{Kur} blockade on atrial repolarization is exquisitely sensitive to vagal stimulation and may be completely abolished in the setting of enhanced parasympathetic neural tone.

Discussion

The management of atrial fibrillation involves the choice of rate control or rhythm control.[4] Clinical trials have demonstrated that for many antiarrhythmic agents, rhythm control does not confer outcomes benefits over rate control,[16-20] but these observations are likely due to poor efficacy and/or toxicity associated with currently available rhythm control options.[17] The challenge presented to researchers is to develop treatments that restore and maintain normal sinus rhythm without the complications that limit widespread use of the currently available methods. In the search for pharmacologic mechanisms that are selective and therefore offer

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greater safety and tolerability compared with available treatments, I_{Kur} blockade is among the most investigated.[8] Several small molecule agents that block I_{Kur} have been in development and have been tested in preclinical settings [21, 22]. In this study, we investigated whether specific inhibition of I_{Kur} might impact on human atrial electrophysiologic properties and hence represent a potential therapeutic target for the prevention of AF.

In preclinical studies, MK-0448 demonstrated a selective inhibition of Kv1.5, the major pore-forming subunit of the native cardiac atrial repolarizing current, I_{Kur}, which is expressed in human atrial tissue, but not in ventricular tissue. This finding is a significant achievement considering that the targets of available therapies, which include I_{Kr} and I_{Ks}, are expressed in ventricular as well as atrial tissue, and thus impact on ventricular refractoriness at therapeutic exposures.

Preclinical studies established a relatively high selectivity for I_{Kur} vs. other cardiac channels; while moderate inhibition of I_{Ks} was detected, there appears to be a 30- to 100-fold window in preclinical experiments between exposures that have a significant and potentially therapeutic effect on atrial refractoriness and exposures that are associated with small but measurable changes in ventricular refractoriness. Preclinical *in vivo* experiments demonstrated selectivity for increasing atrial refractory period at plasma concentrations far below those at which changes in ventricular refractory period were observed. Moreover, studies in conscious dogs with sustained atrial fibrillation in the setting of heart failure demonstrated that in AF terminated in 2 out of 3 dogs after IV administration of MK-0448.

Based on preclinical results demonstrating a selective increase in atrial refractory period, we conducted human studies of MK-0448 to determine safety tolerability, pharmacokinetics and effects on atrial and ventricular electrophysiology. While invasive techniques are not common in

first-in-human settings, this approach allowed for determination of whether MK-0448 had any target effect in the atria, if there is a ventricular effect, and to help guide dose selection for future studies. Indeed, we found only one other study that performed invasive diagnostic electrophysiology evaluation in 23 healthy volunteers.[23] That study also included placement of an intra-arterial catheter for blood pressure monitoring, and the authors did not report on any complications.

MK-0448 was generally well tolerated in our FIH study. However, no increase in atrial or ventricular refractory period was observed despite pharmacologic confirmation of exposure. These results were not expected given the atrial effects observed in preclinical studies. One possible explanation for the difference in preclinical and clinical effects on atrial refractoriness is that elevated vagal tone in the young healthy patients included in the study may have masked or diminished the role of I_{Kur} in atrial repolarization, whereas the preclinical data were obtained in a heart failure model. While tests to definitively determine high vagal tone for the volunteers in this study were not done, the following observations can be made: the subjects were all 23-44 years of age and generally healthy, characteristics that are reliably consistent with high vagal tone [24]; marked respirophasic sinus arrhythmia was noted in all subjects during the EP study, which is a well-known marker of preserved vagal tone. [25, 26]

To examine the possibility that high vagal tone had an effect on our results, we conducted a follow-up study of atrial refractoriness in anesthetized dogs (without heart failure) in the absence and presence of bilateral vagal nerve stimulation. While healthy conscious resting dogs have high vagal tone, our invasive electrophysiology study was performed in anesthetized dogs. In the absence of vagal nerve stimulation, MK-0448 administration resulted in an increase in atrial refractory period, as observed in previous preclinical experiments. In the presence of vagal

nerve stimulation, however, increases in atrial refractory period were greatly attenuated. These results suggest that I_{Kur} blockade by MK-0448 is diminished with enhanced parasympathetic neural tone. The mechanism whereby enhanced vagal tone ameliorates the effect of I_{Kur} blockade on refractory period may involve a shortening of the atrial action potential duration as a result of increasing I_{KACh}.[27] Conversely, it is also possible that MK-0448 is most effective in the presence of enhanced sympathetic tone, as seen in heart failure.

Loss of I_{Kur} blockade by MK-0448 in states of enhanced vagal tone presents a challenge for maintaining therapeutic efficacy, given the purported pro-fibrillatory effects of vagal stimulation. In the dog model, vagal stimulation shortens the atrial ERP in a non-homogeneous manner,[28] so that under states of high vagal tone, the atria demonstrate inhomogeneity of refractoriness. In this milieu, premature impulses will "fractionate" or propagate non-uniformly, setting up reentrant rotors that initiate AF. Withdrawal of vagal tone will often result in cessation of AF in the dog model.[29]

Limitations:

Important limitations of this study are that confounding influences including sympathetic tone, age, and species differences may account for differences between results in dog and human experiments. Indeed there is substantial variability in parameters tested not only between species, but also within species. It is noteworthy that in the full series of heart failure animals with sustained AF tested with two prototype IKur blockers TAEA and ISQ-1, the IKr blocker MK-499 and the Class IC anti-arrhythmic agent propafenone, no test agent was found to be universally effective in terminating AF in all animals tested, suggesting animal-to-animal differences in substrate for AF. Thus the limited numbers of animals tested in this study may

have initially given the impression that MK-0448 may have effectively suppressed atrial fibrillation.

Additionally, our FIH study included young, healthy volunteers, a population that is unlikely to develop AF. Although "lone" AF can occur in younger patients without associated heart disease (often associated with high vagal tone), this is almost invariably a benign and self-terminating arrhythmia. The majority of AF occurs in older patients with coexisting heart disease (hypertension, cardiomyopathy, heart failure, valvular lesions, following thoracic surgery, etc.), and is associated with an elevated risk of stroke. The pathogenesis of these two broad categories of AF has diverse mechanisms, and it remains to be proven that a drug that targets a single channel in healthy hearts would have comparable electrophysiologic and antiarrhythmic effects in diseased hearts.

In summary, extensive preclinical experience with MK-0448 demonstrated that inhibition of I_{Kur} was generally well tolerated and produced increased atrial refractory period without ventricular effects. This study demonstrated a lack of electrophysiologic effects of specific blockade of Kv1.5 in healthy human subjects, as evidenced by a lack of significant effect on atrial refractoriness. This may have been due to diminished contribution of I_{Kur} to atrial repolarization in the presence of enhanced parasympathetic tone; alternately, blockade of Kv1.5 by MK-0448 may only be manifest in the setting of enhanced sympathetic tone. This study provides evidence of the feasibility, value, and safety of diagnostic EP studies in healthy volunteers in early clinical assessments of novel compounds. These data suggest that, depending on the conditions of study including autonomic tone, commonly used preclinical models focusing on atrial electrophysiology may lack clinical translatability. Furthermore, targeting I_{Kur}

may be of limited therapeutic value for the prevention of AF due to sensitivity of effect to parasympathetic tone.

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Table 1. Activity of MK-0448 on Ion Channels

Channel/Subunits		Assay Method	IC_{50}
Human Kv1.5	(n ≥ 3)*	Patch clamp	0.009 μM
Human I_{Kur}	(n =3)	Patch clamp	0.011 μM
I_{Ks} (KCNQ1+KCNE1)	(n ≥ 4)	Patch clamp	0.790 μM
I_{Kr} (hERG)	(n ≥ 3)	Patch clamp	110 μM
I_{Kr} (hERG)	(n =2)	Binding	>30 μM
I_{to} (Kv4.3)	(n ≥ 3)	HT (High Throughput) Clamp	2.336 μM
I_{Na} (SCN5a)	(n ≥ 2)	VIPR (Voltage/Ion Probe Reader)	No effect @ 10 μM
Kv3.2	(n ≥ 2)	HT Clamp	6.074 μM
IK_{Ca}	(n ≥ 3)	Patch clamp	10.2 μM
Kv2.1	(n ≥ 4)	HT Clamp	0.061 μM
Kv1.7	(n ≥ 3)	HT Clamp	0.072 μM
NR1a/2B	(n =2)	FLIPR (Fluorescent Imaging Plate Reader)	6.8 μM

* n refers to the number of measurements at each concentration tested to define the full concentration response curve and thereby determine the IC50; in cases where n ≥ 3, at least 3 observations were made at each concentration, but commonly it might be more, e.g. as many as 6 or 7 at some concentrations, focused on the range between 20 -80 % inhibition, to define the IC50 more precisely

Table 2. Slopes for individual AERP at pacing cycle lengths of 400 ms and 600 ms and VERP 400 and 600 vs. plasma concentration of MK-0448

PD	**Parameters**	**Estimate (95% CI)**	Residual error variance($msec^2$)
AERP400	Intercept	186.16 (169.32, 203.01)	
	Slope	0.0035 (-0.0025, 0.0094)	49.35
AERP600	Intercept	202.76 (173.51, 232.02)	
	Slope	0.0004 (-0.0087, 0.0096)	117.25
VERP400	Intercept	209.22 (191.93, 226.51)	
	Slope	0.0022 (-0.0037, 0.0081)	48.45
VERP600	Intercept	227.40 (204.78, 250.01)	
	Slope	0.0042 (-0.0031, 0.0114)	72.92

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Figure Legends:

Figure 1. Effect of MK-0448 on Recombinant hKv1.5 in Conventional Patch Clamp Experiments (IC_{50} Values). MK-0448 targeted Kv1.5 with an IC_{50} of 8.6 nM (at 3 Hz and 28.0 nM at 1 Hz) for recombinant human Kv1.5 (hKv1.5) heterologously expressed in CHO cells.

Figure 2. Integrated PK-PD plot of MK-0448 plasma concentration versus change in ARRP and VRRP from experiments in anesthetized dogs. The vertical dashed lines indicate the magnitude of the window between the exposures that selectively affect the atrium versus exposures that affect both atrium and ventricle.

Figure 3. Sample electrocardiographic tracings from dog heart failure model and effect of treatment with MK-0448. Panels A, B, and C show readings for placebo before and Panels D, E, and F show readings for MK-0448. Panels A and D show baseline readings before induction of AF. Panels B and E show readings after induction of AF and before dosing. Panels C and F show the effect of medication after dosing. In Panel F conversion from AF to sinus rhythm is observed.

Figure 4. Scatter plots for observed individual AERP at pacing cycle lengths of 400 ms (Panel A) and 600 ms (Panel B) and VERP 400 (Panel C) and 600 (Panel D) vs. plasma concentration of MK-0448 with associated linear regression lines. No significant relationship was exhibited between AERP or VERP at either of the analyzed pacing cycle lengths and drug concentration.

Figure 5. Percentage change in atrial relative refractory period at 30 min (% change from baseline at indicated vagal nerve pacing frequency) in dogs with vagal nerve stimulation