In Vivo Antiarrhythmic and Cardiac Electrophysiologic Effects of a Novel Diphenylphosphine Oxide $i_{\text{Kur}}$ Blocker (2-Isopropyl-5-methylcyclohexyl) Diphenylphosphine Oxide

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ABSTRACT
The antiarrhythmic efficacy of the novel ultrarapid delayed rectifier potassium current ($i_{\text{Kur}}$) blocker (2-isopropyl-5-methylcyclohexyl) diphenylphosphine oxide (DPO-1) was compared with efficacies of the standard class III rapidly activating component of delayed rectifier potassium current ($i_{\text{Kr}}$) blockers $\pm$N-[1’-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxy-spiro[2H-1-benzopyran-2,4’-piperidin]-6-yl) methanesulfonamide hydrochloride (MK499) and ibutilide (two/one preparations; 0.001 mg/kg i.v.) sim-ilarly increased atrial relative (+23.2 and +25.1%, respectively) and effective (+21.6 and +31.9%, respectively) refractory periods. However, antiarrhythmic doses of MK499 and ibutilide also consistently and significantly increased ventricular relative (+9.9 and +7.6%, respectively) and effective (+10.4 and +9.9%, respectively) refractory periods, rate-corrected ECG QTc (+6.7 and +7.8%, respectively), and paced QT (+7.3 and +8.5%, respectively) intervals. Doses of propafenone that terminated atrial arrhythmia (five/five preparations; 0.94 ± 0.54 mg/kg i.v.) significantly increased ECG QRS interval (+11.1%). These findings support the approach of atrial selective modulation of refractoriness through block of $i_{\text{Kur}}$ for the development of potentially safer and more effective atrial antiarrhythmic agents.

Atrial flutter and atrial fibrillation are the most commonly encountered sustained cardiac arrhythmias in clinical practice, and they are associated with significant morbidity, including increased incidences and severity of stroke and heart failure as well as increased mortality (Stewart et al., 2002; Vidaileet et al., 2002). Reentrant excitation is considered to be an important electrophysiologic mechanism underlying both atrial flutter and fibrillation (Konings et al., 1994; Roithinger and Lesh, 1999). Atrial flutter and fibrillation produce electrical remodeling of the human atrium, resulting in a shortening of atrial action potential duration, which in turn enhances the substrate for more recurrent or persistent reentrant atrial arrhythmia (Franz et al., 1997).

The pharmacologic treatment of atrial arrhythmias is limited by the lack of ion channel target and cardiac chamber selectivity of currently available antiarrhythmic drugs, including both class I sodium channel and class III potassium channel blocking agents. This contributes to a high frequency of adverse effects, most notably ventricular proarrhythmia (Choudhury and Lip, 2004; VerNooy and Mounsey, 2004). Atrial selective therapy has been cited as a potential approach for the development of safer and more effective atrial antiarrhythmic agents (Vos, 2005). The ultrarapid delayed rectifier potassium current, $i_{\text{Kur}}$.

ABBREVIATIONS: DPO, diphenylphosphine oxide; DPO-1, (2-isopropyl-5-methylcyclohexyl) diphenylphosphine oxide; CHO, Chinese hamster ovary; MK499, $\pm$N-[1’-6-cyano-1,2,3,4-tetrahydro-2-naphthalenyl]-3,4-dihydro-4-hydroxy-spiro[2H-1-benzopyran-2,4’-piperidin]-6-yl) methanesulfonamide hydrochloride; pQT, paced QT; AET, atrial excitation threshold; VET, ventricular excitation threshold; ECG, electrocardiogram; ARRP, atrial relative refractory period; VRRP, ventricular relative refractory period; AERP, atrial effective refractory period; VERP, ventricular effective refractory period; NIP-142, (3R*, 4S*)-cyclopropylamino-3,4-dihydro-2,2-dimethyl-6-(4-methoxyphenylacetylaminio)-7-nitro-2H-1-benzopyran-3-ol; AVE-0118, 2’-[(2-4-methoxyphenyl)-acetylaminio]-methyl)-biphenyl-2-carboxylic acid (2-pyridin-3-yl-ethyl)-amide; RSD1235, (1R, 2R)-2’-[(3R]-hydroxypropridinyl]-1-(3,4-dimethoxyphenethox)-cyclohexane.
carried by Kv1.5 channels, has been recorded in human atrium but not ventricle (Wang et al., 1993; Amos et al., 1996). As a result, block of $I_{Kur}$ has been proposed as a potential target for atrial selective modulation of refractoriness and treatment of reentrant arrhythmia (Amos et al., 1996; Brendel and Peukert, 2003). Recently, a series of diphenylphosphine oxides (DPOs) represented by the prototype DPO-1 has been shown to block potently human Kv1.5 expressed in CHO cells as well as native $I_{Kur}$ in human atrial myocytes (Lagrutta et al., 2004). In the present study, the antiarrhythmic efficacy and cardiac electrophysiologic effects of DPO-1 were compared with those of the standard class III agents MK499 and ibutilide and the class IC agent propafenone in a canine model of reentrant atrial flutter (Frame et al., 1986). In this head-to-head comparison, doses of DPO-1 that terminated atrial arrhythmia produced a selective increase in atrial refractoriness, whereas effective antiarrhythmic doses of the class III standards affected both atrial and ventricular refractoriness and the ECG QT interval, and the class IC standard increased the ECG QRS interval, all indicative of ventricular effects.

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 (West Point, PA) and 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).

Surgical Preparation. Mongrel dogs ($n = 27$, male or female; $13.7 \pm 22.0$ kg, mean $16.3 \pm 0.5$ kg) were anesthetized with pentobarbital (30 mg/kg i.v.). The animals were intubated and ventilated with room air. The left femoral vein was cannulated for maintenance anesthesia and for test agent administration, and a bilateral vagotomy was performed. A right thoracotomy was performed in the fourth intercostal space, and the pericardium was incised to provide access to the venae cavae and the right atrium. A Y-shaped surgical lesion then was produced as described previously (Frame et al., 1986). Briefly, tissue on a line extending from the superior to inferior venae cavae was clamped, incised, and then sutured together in two stages. An additional incision was made on the center of the right atrial free wall, contiguous with the midpoint of the intercaval lesion, running parallel to the atrioventricular groove and extending toward the base of the right atrial appendage. A stainless steel epicardial bipolar electrode was sutured on the inferior venae cavae adjacent to the origin of right atrial free wall lesion for atrial pacing, and a stainless steel epicardial quadrupolar electrode array was sutured on the right atrial appendage distal to the end of the free wall lesion for the monitoring of atrial flutter rate and for the determination of atrial refractory periods. A stainless steel plunge bipolar electrode was sutured to the right ventricle for the determination of ventricular refractory periods. Following surgical preparation, nadolol (0.5 mg/kg i.v.) and atropine (0.05 mg/kg i.v.) were administered.

Experimental Protocol. Following a 30-min equilibration period, baseline measurements were obtained: sinus heart rate, ECG PR, QRS, and rate-corrected QTc interval ([QT in milliseconds] / [R-R in seconds]), paced QT (pQT) during atrial pacing at 400-ms cycle length, atrial and ventricular excitation thresholds (AET and VET, respectively), relative refractory periods (ARRP and VRRP, respectively), and effective refractory periods (AERP and VERP, respectively) were determined using standard extrastimulus technique during pacing at 400-ms cycle length. Excitation thresholds were defined as the minimum current required to propagate an atrial (AET) or ventricular (VET) extrastimulus of 2-ms pulse duration during diastole. Relative and effective refractory periods were defined as the shortest coupling intervals permitting propagation of an atrial or ventricular extrastimulus at 2-ms pulse duration, introduced initially at 10 ms following by 2-ms decrements, at two times (ARRP and VRRP) and 10 times (AERP and VERP) excitation threshold.

Following baseline measurements, induction of atrial flutter was attempted by rapid right atrial pacing of approximately 3- to 5-s duration starting at a pacing frequency of 6.0 Hz (167-ms cycle length) and increasing by 0.5-Hz increments to a pacing frequency of 20.0 Hz (50-ms cycle length). When atrial flutter was induced, it was monitored for a period of 5 min. Only animals that displayed 5 min of sustained postinduction flutter were entered into the present studies. Animals that displayed sustained flutter were assigned to the following treatments, administered as consecutive i.v. boluses at 5-min intervals: vehicle (polyethylene glycol-200; $n = 6$; four boluses matching volume and timing of test agents), DPO-1 ($n = 6$; 1.0, 3.0, and 10.0 mg/kg) (Aguiar et al., 1976), MK499 ($n = 5$; 0.001, 0.003, and 0.01 mg/kg) (Lynch et al., 1994), ibutilide ($n = 5$; 0.001, 0.003, and 0.01 mg/kg), and propafenone ($n = 5$; 0.1, 0.3, 1.0, and 3.0 mg/kg). In addition to the dedicated vehicle treatment group, all animals assigned to active treatments were administered a single i.v. bolus of vehicle at 5 min of sustained postinduction flutter to further verify that vehicle alone would not terminate flutter. Upon termination of sustained flutter by active test agent or following a brief (1-s) period of right atrial burst pace to terminate flutter in the dedicated vehicle treatment group, post-treatment measurements of sinus heart rate, ECG intervals, and atrial and ventricular excitation and refractory thresholds were determined.

Statistics. Data are expressed as mean ± S.E.M. Comparisons across treatment groups were performed using an analysis of variance, followed by Fisher's protected least significant difference post hoc test when indicated. Within-group pretreatment versus post-treatment comparisons were performed using a paired Student's $t$ test. Post-treatment values were derived at 1 min post-termination following the dose of active test agent terminating flutter in each individual animal, or at 1 min post-termination following brief right atrial burst pacing to terminate flutter in the vehicle group.

Results

In total, 27 animals displaying sustained 5-min postinduction atrial flutter were entered into the present study. Pacing frequency for induction of atrial flutter in this cohort was $9.9 \pm 0.8$ Hz, and it did not vary significantly among the individual treatment groups. Atrial flutter cycle length measured at 5 min postinduction (i.e., before first treatment administration) was $137 \pm 3$ ms and also did not vary significantly among the individual treatment groups.

Table 1 summarizes pretreatment (baseline) versus post-treatment heart rate, ECG interval, atrial and ventricular excitation threshold, and refractory period data, expressed as absolute values, in the individual treatment groups. Figure 1 compares the effects of vehicle and active test agents on ECG QRS interval, rate-corrected QTc interval, and pQT, AERP, and VRRP, expressed as a percentage of change from pretreatment baseline value to normalize comparisons for differences in baseline values among groups as well as differences in baseline values among different parameters.

In the dosage ranges tested, all active test agents were completely effective in terminating sustained atrial flutter. Active test agent doses producing flutter termination were as follows: DPO-1 (six/six preparations terminated at 5.5 ± 2.0 mg/kg i.v.), MK499 (five/five terminated at 0.004 ± 0.002 mg/kg i.v.), ibutilide (five/five terminated at 0.003 ± 0.001 mg/kg i.v.), and propafenone (five/five terminated at 0.94 ± 0.54 mg/kg i.v.). Figure 2 depicts a study in which DPO-1 terminated sustained atrial flutter. In the dedicated vehicle treatment group, four successive i.v. bolus administrations of...
vehicle failed to terminate atrial flutter in all six animals tested. Likewise, in all active test agent treatment groups, single i.v. boluses of vehicle administered at 5 min postin-duction failed to terminate flutter in all animals tested.

In the dedicated vehicle treatment group, significant decreases in both ARRP [−10.9% (−16.5 ± 5.3 ms)] and AERP [−12.8% (−15.5 ± 4.1 ms)] were observed following termination of flutter. In addition, a slight decrease in AET and a slight increase in VET, the latter noted in several treatment groups, were observed following flutter termination. There were no significant changes in ECG intervals, including QTc and paced QT intervals, or in ventricular refractory periods in the vehicle group.

At doses terminating atrial flutter, the predominant cardiac electrophysiologic effects of DPO-1 were significant increases in both ARRP [+15.7% (+21.0 ± 5.7 ms) and +15.2% (+16.3 ± 9.9 ms), respectively]. No changes in VRRP, VERP, ECG QTc, and paced QT intervals were observed at the effective antiarrhythmic doses of DPO-1. A slight increase in VET was noted following DPO-1 administration, as was also noted in the vehicle treatment group.

MK499 and ibutilide displayed similar electrocardiographic and cardiac electrophysiologic profiles at doses terminating atrial flutter. Both MK499 and ibutilide increased ARRP [23.2% (+32.0 ± 9.7 ms) and 25.1% (+37.2 ± 8.3 ms), respectively] and AERP [21.6% (+25.0 ± 7.0 ms) and 31.9% (+40.0 ± 6.3 ms), respectively], but they also significantly increased VRRP [9.9% (+15.4 ± 5.1 ms) and 7.6% (+12.2 ± 3.1 ms), respectively] and VERP [10.4% (+13.8 ± 4.3 ms) and 9.9% (+13.4 ± 4.0 ms), respectively]. Consistent with the increases in ventricular refractory periods, MK499 and ibutilide significantly increased ECG QTc [6.7% (+22.6 ± 3.2 ms) and 7.8% (+24.0 ± 7.2 ms), respectively] and paced QT intervals [7.3% (+17.8 ± 6.2 ms) and 8.5% (+21.0 ± 4.8 ms), respectively] at effective antiarrhythmic doses. Both compounds variably tended to increase VET.

At doses terminating atrial flutter, the predominant effects of propafenone were a significant decrease in heart rate and a significant [11.1% (+7.4 ± 1.1 ms)] increase in ECG QRS interval. Modest but not statistically significant increases in ARRP and AERP as well as AET and VET also were observed at effective doses of propafenone.

**Discussion**

In the present study, a canine model of atrial flutter was used to demonstrate termination of reentrant atrial arrhythmia with the I_{Kur} blocker DPO-1, the class III standards
MK499 and ibutilide, and the class IC standard propafenone. In contrast to effective doses of the class III agents, which prolonged ventricular refractory periods and ECG QT intervals, and the class IC agent, which prolonged ECG QRS interval, doses of DPO-1 that terminated atrial arrhythmia elicited selective increases in atrial refractory periods. Although controversial, recent evidence indicates that dog atrium possesses Kv1.5 protein and functionally important

**Fig. 2.** Termination of atrial flutter after administration of DPO-1. For each panel, top trace is lead II ECG and bottom trace is left atrial electrogram (LA-EG). A, sinus rhythm before induction of atrial flutter. B, induction of flutter by right atrial burst pacing at a rate of 8.5 Hz. C, 1 min after vehicle administration. D, 1 min after 1.0 mg/kg i.v. DPO-1. E, 1 min after 3.0 mg/kg i.v. DPO-1. F, termination of atrial flutter at 1 min 30 s after 10.0 mg/kg i.v. DPO-1. G, sinus rhythm at 1 min following flutter termination.
I_{Kur} (Fedida et al., 2003), indicating dog to be a physiologically appropriate test species for the study of I_{Kur} blockade.

The canine model of Y-shaped intracaval and right atrial free wall surgical lesions (Frame et al., 1986) permits the induction of sustained atrial flutter, stable within animal and comparable among animals. Atrial flutter in this model has been characterized as reentrant, using two conduction barriers, the tricuspid annulus for reentry and the surgical lesion to protect the reentrant circuit from excitation by wave fronts other than the reentrant wave front (Frame et al., 1987; Boyden et al., 1989). This model possesses similarity to typical human flutter, which also has been mapped as reentrant, using the tricuspid annulus as an anterior barrier and the crista terminalis and Eustachian ridge as the posterior "protective" barrier (Roithinger and Lesh, 1999). Due to its consistent nature, the Y-shaped atrial lesion model has been used extensively to study the properties of atrial reentrant excitation and antiarrhythmic drug efficacy.

Class IA and IC sodium channel blocking agents tested previously in the canine Y-shaped atrial lesion model, including procainamide, quinidine, flecainide, and propafenone, terminated atrial flutter and produced a greater slowing of conduction and increases in flutter cycle length compared with changes in refractoriness (Wu and Hoffman, 1987; Spinelli and Hoffman, 1989; Wu et al., 1989a). It has been postulated that these agents terminate reentry by depressing conduction to a point where propagation fails (Spinelli and Hoffman, 1989). The class IB agents lidocaine and phenytoin, in contrast, produced only modest slowing of conduction and were poorly effective in termination (Spinelli and Hoffman, 1989; Wu et al., 1989a).

Early class III potassium channel blocking agents, including N-acetylprocainamide, clofilium, and d-sotalol, the latter predominantly a blocker of the rapidly activating component of delayed rectifier potassium current, I_{Kr} (Sanguinetti and Jurkiewicz, 1990), terminated atrial arrhythmia in this model while producing greater increases in atrial refractoriness compared with slowing of conduction (Wu and Hoffman, 1987; Spinelli and Hoffman, 1989; Wu et al., 1989b). The modest slowing of flutter observed with class III agents has been attributed to increased refractoriness in the partially repolarized excitable gap, resulting in advancement of the reentrant wave front into less fully repolarized cells, thereby propagating at a reduced velocity (Wu et al., 1989b). Termination by a class III agent would then occur when propagation could slow no more because the tissues in advance of the wave front were too refractory to generate a propagated response (Wu et al., 1989b). Additionally, failure of the lateral conduction boundaries of the reentrant circuit as well as reflection and collision within the reentrant path have been described as alternate mechanisms underlying termination of reentrant atrial arrhythmia with class III agents in this model (Spinelli and Hoffman, 1989). Ibutilide, a class III agent reported to enhance inward sodium current (Lee, 1992) but also shown to block I_{Kr} potently (Yang et al., 1995), terminated atrial flutter in this model with attendant increases in atrial refractoriness and increased flutter cycle length (Buchanan et al., 1995).

In the present study, vehicle alone failed to terminate atrial flutter, demonstrating that flutter termination in active treatment groups was test agent-dependent as opposed to a stochastic event. In the dedicated vehicle treatment group, atrial refractory periods following the termination of sustained flutter were decreased significantly compared with pretreatment baseline. Decreases in atrial refractoriness following relatively brief periods of either rapid atrial pacing or atrial fibrillation have been reported in other dog models (Miyata et al., 2002; Watanabe et al., 2003) and in human (Daoud et al., 1996; Yu et al., 1998). This decrease in atrial refractoriness following even relatively brief periods of rapid atrial rate constitutes a component of “electrical remodeling” that predisposes the atrium to more frequent or persistent reentrant atrial arrhythmia. The effects of test agents on atrial refractoriness in this atrial flutter termination model must be viewed in the context of significant decreases in atrial refractoriness in the absence of active treatment.

The class IC agent propafenone and the class III agents ibutilide and MK499, the latter a potent and selective I_{Kr} blocker (Lynch et al., 1994), were included in the present study as positive standards. Consistent with previous experience in this model with either specific compounds or mechanistic class, all three standard agents terminated atrial flutter. Effective termination doses of MK499 and ibutilide were associated not only with marked increases in atrial refractoriness but also with consistent, significant increases in ventricular refractory periods and ECG QT intervals. Effective termination doses of propafenone were associated with increased ECG QRS interval, indicative of slowing of ventricular conduction, with only modest increases in atrial refractoriness.

The lack of atrial versus ventricular selectivity displayed in the present study by the class III I_{Kr} blockers MK499 and ibutilide and the class IC agent propafenone illustrates a significant limitation of currently available atrial antiarrhythmic agents. Currently available agents are associated with a high incidence of adverse effects, most notably the occurrence of ventricular proarrhythmia, which limits their efficacy and utility (Choudhury and Lip, 2004; VerNooy and Mounsey, 2004). Atrial selective therapy, such as through block of I_{Kur}, has gained attention as a potential mechanism for safer and more effective atrial antiarrhythmic agents (Brendel and Peukert, 2003; Vos, 2005). Several compounds have been reported to block I_{Kur} as part of a spectrum of ion channel activities and to suppress atrial arrhythmias in animal models, including NIP-142 (Nagasawa et al., 2002), AVE-0118 (Knobloch et al., 2004; Blauw et al., 2004), and RSD1235 (Beatch et al., 2002, 2004). However, the aforementioned compounds are relatively nonselective, displaying no greater than 3-fold selectivity for Kv1.5 and I_{Kur} over other cardiac potassium channels, including I_{Kr}, transient outward potassium current (I_{to}), and G protein-regulated, muscarinic-activated potassium current (I_{K(ACh)}) (Matsuda et al., 2001; Seki et al., 2002; Beatch et al., 2003; Brendel and Peukert, 2003; Gogelein et al., 2004).

DPO-1 is representative of a novel DPO I_{Kur} blocker pharmacophore. The cellular electrophysiologic activities of DPO analogs have been assessed in CHO cells stably transfected with human Kv1.5, isolated human atrial myocytes, and guinea pig ventricular myocytes (Lagrutta et al., 2004). In CHO cells, DPO-1 inhibited human Kv1.5 current in a forward frequency-dependent manner, with IC_{50} values of 0.16, 0.05, and 0.03 µM at pacing frequencies of 0.1, 1.0, and 3.0 Hz, respectively. Similarly, DPO-1 inhibited I_{Kur} in isolated human atrial myocytes with IC_{50} values of 0.08, 0.04, and...
0.03 μM at pacing frequencies of 0.1, 1.0, and 3.0 Hz, respectively, at a test concentration of 1.0 μM, DPO-1 failed to inhibit I\textsubscript{K1.5} in isolated human atrial myocytes. In isolated guinea pig ventricular myocytes, DPO-1 at a test concentration of 3.0 μM elicited minimal to no block of other cardiac K\textsuperscript{+} currents: 15% block of inward rectifier potassium current (I\textsubscript{Kir}), 3% block of I\textsubscript{K1.5}, and 25% block of slowly activating component of delayed rectifier potassium current (I\textsubscript{Kur}) (Lagrutta et al., 2004).

In the present studies, DPO-1 effectively terminated reentrant atrial arrhythmia in the Y-shaped atrial lesion model. In contrast to the profiles described above for MK499, ibutilide, and propafenone, doses of DPO-1 terminating atrial arrhythmia were associated with selective increases in atrial refractoriness, and no concomitant effects on ventricular refractoriness or conduction. These observations support the approach of atrial selective modulation of refractoriness through block of I\textsubscript{Kur} for the development of potentially safer and more effective antiaarrhythmic agents. Several elements of experimental design are important in the interpretation of the present results. Most importantly, it should be noted that this study was designed to assess cardiac electrophysiologic effects at those doses of test agents terminating reentrant atrial arrhythmia in this animal model. It is possible that at doses higher than those required to terminate arrhythmia, the atrial versus ventricular selectivities of the test agents will vary from those observed in the present study. Similarly, the reentrant arrhythmia induced in the present animal model was characterized by a relatively uniform 137 ± 3 ms cycle length, and refractory period determinations in this study were conducted at a pacing cycle length of 400 ms. Alterations in these conditions may alter both the efficacy and cardiac electrophysiologic profiles of the test agents. Explorations of the aforementioned issues constitute logical extensions of preclinical evaluation. Finally, additional preclinical studies required to advance the concept further of atrial selective modulation of refractoriness through I\textsubscript{Kur} blockade include the testing of a selective blocker in an animal model of atrial arrhythmia occurring in a relevant disease state. The latter is particularly important in assessing the effect of I\textsubscript{Kur} blockade in the setting of more sustained atrial arrhythmia, in which mechanical and electrical remodeling may alter efficacy.

References


