Electrophysiologic and Antiarrhythmic Effects of AZD1305 in Canine Pulmonary Vein Sleeves

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ABSTRACT

The objective of this study was to examine the electrophysiologic and antiarrhythmic effects of the new antiarrhythmic agent *tert*-butyl (2-{7-[2-(4-cyano-2-fluorophenoxy)ethyl]-9-oxa-3,7-diazabicyclo[3.3.1]non-3-yl}ethyl)carbamate (AZD1305) in canine pulmonary vein (PV) sleeve preparations isolated from untreated and long-term amiodarone-treated animals. Ectopic activity arising from PV sleeves plays a prominent role in the development of atrial fibrillation (AF). Delayed afterdepolarizations (DADs) and late phase 3 early afterdepolarizations (EADs), originating from the PV have been proposed as potential triggers in initiation of AF. Action potentials were recorded from canine superfused PV sleeves using standard microelectrode techniques. Acetylcholine (1 μ M), isoproterenol (1 μ M), or their combination was used to induce EADs, DADs, and triggered activity (TA). The effects of AZD1305 (0.1–10 μ M) were evaluated in PV sleeve preparations isolated from untreated and

amiodarone-treated (40 mg/kg daily for 6 weeks) dogs. AZD1305 (0.1–10 μ M, 30 min) significantly prolonged action potential duration and reduced excitability. Abbreviating basic cycle length from 1000 to 300 ms resulted in a decrease of $V_{\max}$ from 314 ± 79 to 251 ± 55 V/s ($\Delta = -20\%$) in control and from 177 ± 53 to 76.5 ± 33 V/s ($\Delta = -57\%$) after AZD1305 ($n = 6$, $p < 0.05$). AZD1305 markedly attenuated or suppressed DADs and DAD-induced TA, but not late phase 3 EADs. AZD1305-induced attenuation of excitability, leading to activation failure at much longer cycle lengths, was much more pronounced in PV from amiodarone-treated dogs. Potent effects of AZD1305 to depress excitability, prolong action potential duration, and suppress DAD-induced triggered activity in canine PV sleeve preparations may be effective in suppressing triggers responsible for the genesis of AF and other atrial arrhythmias.

Clinical and experimental studies have shown that arrhythmogenic activities originating from pulmonary veins (PVs) (Haïssaguerre et al., 1998) are potential triggers in the initiation of AF (Patterson et al., 2005, 2006; Burashnikov and Antzelevitch, 2006; Lo et al., 2007; Wongcharoen et al., 2007).

tert-Butyl (2-{7-[2-(4-cyano-2-fluorophenoxy)ethyl]-9-oxa-3,7-diazabicyclo[3.3.1]non-3-yl}ethyl)carbamate (AZD1305) (Fig. 1) is a combined ion channel blocker that has undergone clinical testing for restoration and maintenance of sinus rhythm in patients with AF. In vitro studies in transfected mammalian cells and ventricular cardiomyocytes show that AZD1305

predominantly blocks the rapid component of the delayed rectifying potassium current (I_{Kr}), the L-type calcium current, and the inward sodium current, actions that all contribute to its functional effects on action potential duration, refractoriness, and conduction (Carlsson et al., 2009; Andersson et al., 2010). This ion channel-blocking profile seems to translate into atrial predominant electrophysiologic actions as well as a low potential for ventricular proarrhythmia such as torsades de pointes (Burashnikov et al., 2010; Carlsson et al., 2009; Andersson et al., 2010). Amiodarone is commonly used for the treatment of AF and is probably the most effective pharmacologic option for maintenance of sinus rhythm. The objective of the present study was 2-fold. First, the study was designed to evaluate the electrophysiologic and antiarrhythmic effects of AZD1305 in canine superfused PV sleeve preparations isolated from untreated dogs. Second, preparations isolated from dogs treated long-term with amiodarone were studied to assess potential additive or synergistic elec-

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ABBREVIATIONS: PV, pulmonary vein; AF, atrial fibrillation; AZD1305, *tert*-butyl (2-{7-[2-(4-cyano-2-fluorophenoxy)ethyl]-9-oxa-3,7-diazabicyclo[3.3.1]non-3-yl}ethyl)carbamate; BCL, basic cycle length; TOP, takeoff potential; APA, action potential amplitude; APD₅₀, action potential duration at 50% repolarization; APD₉₀, action potential duration at 90% repolarization; ACh, acetylcholine; EAD, early afterdepolarization; DAD, delayed afterdepolarization; TA, triggered activity; AZD7009, *tert*-butyl-2-(7-[(2S)-3-(4-cyanophenoxy)-2-hydroxypropyl]-9-oxa-3,7-diazabicyclo[3.3.1]non-3-yl)ethylcarbamate).

Fig. 1. Molecular structure of AZD1305.

trophysiologic actions of the two compounds. Preliminary data have been presented in abstract form (Sicouri et al., 2008b).

Materials and Methods

This investigation conforms to the *Guide for Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) and was approved by the Animal Care and Use Committee of the Masonic Medical Research Laboratory.

Six adult mongrel dogs were orally treated with amiodarone (40 mg/kg/day) for 6 weeks. The dose of amiodarone used is considerably higher than the regimen typically used clinically for management of AF but falls within the range of doses previously used in dog studies (Gallagher et al., 1989; Abdollah et al., 1990; van Opstal et al., 2001). Serum concentrations of amiodarone measured using these high dosage regimens (Sicouri et al., 1997) fell within the clinically effective concentration range (Holt et al., 1986), supporting the appropriateness of the high oral dosing. No signs of hypothyroidism (marked bradycardia, lethargic state, and lack of appetite), hyperthyroidism (tachycardia and excitation), or lung toxicity (breathing disability) were observed over the course of the study.

In the present study, untreated dogs or dogs treated long-term with amiodarone for 6 weeks were anticoagulated with heparin (180 IU/kg) and anesthetized with sodium pentobarbital (35 mg/kg i.v.). The chest was opened via a left thoracotomy, and the heart was excised and placed in a cold cardioplegic solution ($[K^+]_o = 8$ mM, 4°C).

Superfused Pulmonary Vein Sleeve Preparation. PV sleeve preparations (approximately 2.0×1.5 cm) were isolated from left canine atria. The thickness of the preparation was approximately 2 mm. Left superior PVs were used preferentially in most experiments. The preparations were placed in a small tissue bath and superfused with Tyrode's solution of the following composition: 129 mM NaCl, 4 mM KCl, 0.9 mM NaH_2PO_4 , 20 mM NaHCO_3 , 1.8 mM CaCl_2 , 0.5 mM MgSO_4 , and 5.5 mM glucose, buffered with 95% O_2 -5% CO_2 ($35 \pm 0.5^\circ\text{C}$). The PV preparations were stimulated at a basic cycle length (BCL) of 1000 ms during the equilibration period (1 h) using electrical stimulation (1–3-ms duration, 2.5 times diastolic threshold intensity) delivered through silver bipolar electrodes insulated except at the tips. Transmembrane potentials were recorded (at a sampling rate of 41 kHz) using glass microelectrodes filled with 3 M KCl (10–20 M Ω d.c. resistance) connected to a high input impedance amplification system (model KS-700; World Precision Instruments, New Haven, CT). The following parameters were measured: take-off potential (TOP), action potential amplitude (APA), action potential duration at 50 and 90% repolarization (APD_{50} and APD_{90}), maximal rate of rise of action potential upstroke (V_{max}), and the minimal cycle length required to maintain 1:1 activation. The TOP was used instead of the resting membrane potential because of the slow phase 3 of the action potential of the PV sleeve preparation, which does not return to the resting potential at the shortest BCLs. Acetylcholine (ACh) (1 μM), isoproterenol (1 μM), or their combination were used to induce late phase 3 early afterdepolarizations (EADs), delayed

afterdepolarizations (DADs), and triggered activity. The combination of parasympathetic and sympathetic stimulation has been shown to facilitate the development of late phase 3 EADs in PV sleeve preparations (Patterson et al., 2005, 2006; Burashnikov and Antzelevitch, 2006), whereas sympathetic stimulation is known to lead to calcium overload, a condition responsible for the development of DADs (Chen et al., 2000; Chen and Chen, 2006). DADs or EADs were elicited using stimulation trains of 20 beats introduced at progressively faster rates followed by a pause.

Drugs. Amiodarone (Cordarone, 200-mg tablet) was obtained from Wyeth Pharmaceuticals (Vonnore, TN). AZD1305 (AstraZeneca R&D, Mölndal, Sweden) was used at concentrations of 0.1 to 10.0 μM , a concentration range covering anticipated effective plasma concentrations for restoration and maintenance of sinus rhythm in patients (0.5–3 μM).

Statistics. Results are presented as means $\pm$ S.D. Statistical analysis was performed using one-way repeated-measures analysis of variance followed by Bonferroni's test. Mean values were considered to be different at $p < 0.05$.

Results

Effects of AZD1305 on Action Potential Characteristics of Pulmonary Vein Sleeve Preparations. Figure 2 shows a representative example of the effects of AZD1305 (10 μM) on action potential morphology and V_{max} of a PV sleeve preparation. AZD1305 induced a marked use-dependent decrease in V_{max} and 2:1 activation failure at a BCL of 200 ms. Composite data ($n = 6$) of the effects of AZD1305 on V_{max} are shown in Fig. 3 and Table 1. AZD1305 (3 and 10 μM) induced a significant decrease in V_{max} , APA, and TOP and a significant increase in APD_{90} . The effects of AZD1305 were more pronounced at the faster rates of stimulation (use-dependent effect) and at higher concentration of AZD1305.

Effects of AZD1305 on Excitability. Figure 4 illustrates composite data of the effects of AZD 1305 on the BCL at which 1:1 activation was lost ($n = 5$). AZD1305 (3 and 10 μM) induced a significant increase of the BCL required to maintain 1:1 capture, indicating that the drug markedly depressed excitability.

Effect of AZD1305 on DADs and EADs. In another series of experiments we investigated the effects of AZD1305 on EADs, DADs, and TA provoked by isoproterenol in PV

Fig. 2. Effects of AZD1305 on action potential morphology and maximum rate of rise of action potential upstroke (V_{max}) in a PV sleeve preparation. Top panel, control recordings. Bottom, effect of AZD1305 (10 μM). BCL: 1000, 300, and 200 ms. AZD1305 induces a marked use-dependent decrease in V_{max} and 2:1 activation failure at BCL 200 ms.

Fig. 3. Composite data of the effects of AZD1305 on action potential parameters of PV sleeve preparations ($n = 6$). A, effects on the maximal rate of rise of the action potential upstroke ($V_{\max}$). B, effects on APD_{90} . C, effects on APA. D, effects on TOP. AZD1305 (3 and 10 μM) causes a significant decrease in $V_{\max}$, APA, and TOP and significantly increases APD_{90} . *, $p < 0.05$, AZD1305 versus control.

TABLE 1

Effects of AZD1305 on APD_{50} and APD_{90} , $V_{\max}$, APA, and TOP in PV sleeve preparations at BCLs of 1000, 500, 300, and 200 ms $n = 6$.

	APD_{90}	APD_{50}	$V_{\max}$	APA	TOP
	ms		V/s	ms	mV
BCL = 1000 ms					
Control	200.7 ± 25.6	68.7 ± 17.1	314.0 ± 79.2	113.7 ± 3.2	-85.3 ± 2.3
0.1 μM	198.0 ± 22.9	68.2 ± 17.0	307.3 ± 81.7	113.7 ± 3.2	-85.3 ± 2.3
0.3 μM	199.2 ± 23.8	69.7 ± 16.8	301.7 ± 82.1	113.5 ± 3.1	-85.3 ± 2.3
1.0 μM	217.8 ± 48.4	68.8 ± 17.4	288.3 ± 1.7	112.0 ± 3.8	-84.5 ± 1.6
3.0 μM	$228.0 \pm 56.8^*$	66.3 ± 18.3	$244.5 \pm 62.1^*$	$105.8 \pm 5.2^*$	-83.0 ± 1.1
10.0 μM	$232.0 \pm 46.3^*$	70.0 ± 14.8	$177.83 \pm 53.4^*$	$98.0 \pm 5.4^*$	-82.2 ± 0.8
BCL = 500 ms					
Control	172.2 ± 26.5	65.5 ± 15.6	294.3 ± 67.9	112.3 ± 3.1	-84.2 ± 2.6
0.1 μM	170.8 ± 27.1	65.2 ± 15.2	289.7 ± 68.0	112.3 ± 3.1	-84.2 ± 2.6
0.3 μM	173.5 ± 27.1	66.0 ± 15.8	279.5 ± 65.1	112.3 ± 3.1	-84.2 ± 2.6
1.0 μM	186.8 ± 32.7	66.7 ± 17.2	255.3 ± 66.0	110.2 ± 4.2	-82.8 ± 1.7
3.0 μM	$197.2 \pm 45.0^*$	67.0 ± 17.2	$197.33 \pm 50.3^*$	$102.2 \pm 4.1^*$	$-81.2 \pm 0.8^*$
10.0 μM	$208.0 \pm 45.2^*$	68.5 ± 13.6	$130.16 \pm 47.5^*$	$92.7 \pm 6.1^*$	$-79.8 \pm 0.8^*$
BCL = 300 ms					
Control	146.0 ± 21.2	62.2 ± 14.1	251.0 ± 55.1	108.3 ± 4.4	-82.2 ± 2.9
0.1 μM	146.3 ± 20.0	61.8 ± 14.2	251.0 ± 56.3	108.3 ± 4.4	-82.0 ± 3.1
0.3 μM	147.2 ± 21.2	62.8 ± 14.4	235.7 ± 43.5	108.2 ± 4.4	-82.0 ± 3.1
1.0 μM	161.5 ± 29.3	60.7 ± 15.6	$185.5 \pm 36.9^*$	103.0 ± 6.5	-79.8 ± 1.9
3.0 μM	$184.8 \pm 36.7^*$	63.8 ± 17.6	$137.83 \pm 43.9^*$	$95.7 \pm 7.5^*$	$-78.7 \pm 1.5^*$
10.0 μM	$182.2 \pm 26.7^*$	65.0 ± 14.5	$76.5 \pm 33.1^*$	$85.0 \pm 5.1^*$	$-76.8 \pm 1.3^*$
BCL = 200 ms					
Control	127.7 ± 8.1	54.3 ± 11.1	175.3 ± 52.1	99.8 ± 7.7	-79.3 ± 3.6
0.1 μM	126.7 ± 8.8	53.8 ± 10.6	175.0 ± 52.0	99.7 ± 7.8	-79.3 ± 3.6
0.3 μM	128.2 ± 8.5	54.5 ± 11.0	$166.5 \pm 54.4^*$	101.2 ± 6.9	-81.7 ± 6.7
1.0 μM	130.5 ± 15.6	53.0 ± 13.6	$103.66 \pm 58.4^*$	92.5 ± 9.4	$-76.2 \pm 3.0^*$
3.0 μM	129.8 ± 24.5	41.0 ± 15.5	$65.16 \pm 47.7^*$	$81.7 \pm 12.1^*$	$-73.2 \pm 5.8^*$
10.0 μM	Activation failure				

* $p < 0.05$, AZD1305 versus control.

sleeve preparations. A representative experiment of the effects on DAD-induced triggered activity is shown in Fig. 5. Isoproterenol-induced DAD activity was apparent after a train of 20 beats at BCLs of 300 and 150 ms. The DAD reached a threshold at a BCL of 120 ms, giving rise to a triggered response followed by a prominent DAD. AZD1305 (3 μM) suppressed the triggered response and reduced the amplitude of the DADs. DAD-induced triggered activity was suppressed by 3 μM AZD1305 in five of five preparations. Late phase 3 EADs induced by ACh or a combination of ACh and isoproterenol were largely unaffected by AZD1305 ($n = 3$).

Effect of AZD1305 on Long-Term Amiodarone-Treated PV Sleeve Preparations. Figure 6 illustrates composite data of the effects of AZD1305 on the BCL at which 1:1 activation

failed in PV sleeves isolated from untreated and amiodarone-treated dogs. In untreated animals AZD1305 (3 and 10 μM) led to a significant increase of the BCL required to maintain 1:1 activation, from 124 ms under control conditions to 165 and 246 ms after 3 and 10 μM AZD1305, respectively. In preparations from long-term amiodarone-treated dogs, AZD1305 (1 μM) dramatically increased the BCL required to maintain 1:1 activation, from 420 ms with long-term amiodarone alone to 1060 ms after the addition of 1 μM AZD1305. Thus, in PV sleeve preparations, pretreatment with long-term amiodarone potentiates the effect of AZD1305 to depress excitability, suggesting a synergistic effect of AZD1305 and long-term amiodarone. In long-term amiodarone-treated PV preparations, late phase 3 EAD- and DAD-induced triggered activity was rarely observed in the

Fig. 4. Effect of AZD1305 on the briefest BCL at which 1:1 activation is maintained in the PV sleeve preparations ($n = 5$). AZD1305 (3 and 10 μM) significantly increases the BCL at which 1:1 is maintained. *, $p < 0.05$ AZD1305 versus control.

presence of isoproterenol and/or ACh (even at higher concentrations). Hence, the effect of AZD1305 on EADs and DADs could not be adequately tested because of the protective effect of long-term amiodarone.

Discussion

Our results indicate that in PV sleeve preparations, AZD1305 exerts potent concentration-dependent effects to depress excitability, prolong action potential duration, and suppress DAD-induced triggered activity. We observed a significant use-dependent decrease in V_{max} , action potential amplitude, and take-off potential and a significant increase in action potential duration. These actions of AZD1305 are presumably secondary to its frequency-dependent blockade of the fast sodium current and the I_{Kr} current as demonstrated in mammalian cell lines expressing the human isoforms of the ion channel proteins (Carlsson et al., 2009). In addition, the data show that in the PV sleeves isolated from the long-term amiodarone-treated dogs, AZD1305 acted synergistically to further depress excitability.

The effects of AZD1305 are similar to those described for its analog *tert*-butyl-2-(7-[(2*S*)-3-(4-cyanophenoxy)-2-hydroxypropyl]-9-oxa-3,7-diazabicyclo[3.3.1]non-3-yl)ethylcarbamate (AZD7009), which showed high efficacy in restoring sinus rhythm in patients with long-lasting AF (Carlsson et al., 2006; Geller et al., 2009). In addition, AZD1305 has been shown to attenuate I_{Kr} blockade-induced APD prolongation in rabbit isolated Purkinje fibers and to widen the QRS interval and suppress dofetilide-induced torsades de pointes in anesthetized rabbits (Carlsson et al., 2009; Andersson et al., 2010), actions attributed to its high potency in blocking the L-type calcium current and the late (persistent) sodium current. Accordingly, in the canine left ventricular wedge

Fig. 6. Effect of AZD1305 on the briefest BCL at which 1:1 activation is maintained in PV sleeve preparations isolated from untreated and long-term amiodarone-treated dogs. AZD1305 (3 and 10 μM) causes a significant increase in the BCL required to maintain 1:1 activation. Pretreatment with amiodarone significantly potentiates the effect of AZD1305. *, $p < 0.05$ versus control; #, $p < 0.05$ amiodarone+AZD1305 versus amiodarone alone.

preparation, AZD1305 significantly increased R wave width (a surrogate measure of conduction velocity and sodium current blockade) at the highest concentration tested (10 μM) and slightly delayed repolarization, but spontaneous or programmed electrical stimulation-induced proarrhythmia was never observed (J. M. Di Diego, personal communication).

Effects of AZD1305 on DAD and EAD Activity in PV Sleeves. Previous studies have shown that DAD- and late-phase EAD-induced TA can be easily induced in canine PV sleeve preparations after the addition of isoproterenol, ACh, and high calcium alone or in combination (Patterson et al., 2005, 2006; Sicouri et al., 2008a, 2009), in canine- and rabbit-isolated single PV myocytes (Chen et al., 2000; Chen and Chen, 2006) and in canine coronary perfused right atrial preparations (Burashnikov and Antzelevitch, 2003, 2006). Conditions permitting intracellular calcium loading facilitate the development of DADs and late phase 3 EADs (Burashnikov and Antzelevitch, 2006). The present study shows that AZD1305 is capable of suppressing DAD-induced triggered responses in PV sleeve preparations. However, late phase 3 EADs were largely unaffected by the drug. The effects of AZD1305 on DAD-induced triggered activity are similar to those of ranolazine (Sicouri et al., 2008a).

Synergistic Effects of Amiodarone and AZD1305. In PV sleeve preparations isolated from amiodarone-treated dogs, AZD1305 markedly depresses excitability (Fig. 6). The shortest BCL at which 1:1 activation could be maintained was dramatically increased from 420 to 1060 ms after the addition of low concentration of AZD1305 (1 μM), pointing to a synergistic effect of the drug combination. Similar syner-

Fig. 5. Effect of AZD1305 on DADs and triggered activity induced by isoproterenol in a PV sleeve preparation. Top, effect of isoproterenol (1 μM). DAD activity is apparent after a train of 20 beats at BCL of 300 and 150 ms. The DAD reaches threshold at a BCL of 120 ms, giving rise to a triggered response followed by a prominent DAD. Bottom, AZD1305 (3 μM) suppresses isoproterenol-induced triggered response and reduces the amplitude of the DADs.

gistic use-dependent depression of excitability was recently reported for the combination of chronic amiodarone and acute ranolazine in the canine isolated PV preparation (Sicouri et al., 2010). Furthermore, the drug combination effectively suppressed triggered activity in the PV sleeve preparations.

The synergism of chronic amiodarone and AZD1305 may be due to their interaction with different states of the cardiac sodium channel. Amiodarone is an inactivated state blocker of cardiac sodium channels (Whalley et al., 1995; Kodama et al., 1999). If AZD1305 is a predominantly activated state blocker of the sodium channel, the drug combination could lead to a synergistic depression of I_{Na} by blocking the channel during both activated and inactivated states. In support of this hypothesis, our preliminary voltage clamp data indicate that AZD1305 interacts most strongly with the open or activated state of the sodium channel (Burashnikov et al., 2010). A synergistic electrophysiologic action between amiodarone and a combined ion channel blocker such as AZD1305 may be effective in suppressing AF, as has been demonstrated for the combination of amiodarone and ranolazine (Antzelevitch et al., 2009). However, before embarking on such studies it has to be demonstrated that any potential drug combination does not exacerbate the risk of repolarization delay and torsades de pointes. Whether amiodarone-treated patients can be safely converted with any repolarization-delaying agent is still unclear. A few clinical studies have demonstrated that restoration of sinus rhythm by ibutilide in patients receiving amiodarone is as safe (and effective) as the use of ibutilide alone (Glatter et al., 2001; Fragakis et al., 2005). In the canine left ventricle, long-term treatment with amiodarone differentially altered the cellular electrophysiology of the ventricular myocardium such that the transmural dispersion of repolarization decreased (Sicouri et al., 1997). Furthermore, amiodarone dramatically decreased the effect of *d*-sotalol to exaggerate dispersion of repolarization or to induce repolarization-related proarrhythmia.

In conclusion, the actions of AZD1305 to depress excitability, prolong action potential duration, and eliminate TA in PV sleeve preparations may be effective in suppressing the triggers responsible for the development of AF and other atrial arrhythmias.

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