Human Electrophysiological and Pharmacological Properties of XEN-D0101: A Novel Atrial-Selective Kv1.5/I_{Kur} Inhibitor

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Abstract: The human electrophysiological and pharmacological properties of XEN-D0101 were evaluated to assess its usefulness for treating atrial fibrillation (AF). XEN-D0101 inhibited Kv1.5 with an IC_{50} of 241 nM and is selective over non-target cardiac ion channels (IC_{50} Kv4.3, 4.2 μM; hERG, 13 μM; activated Nav1.5, >100 μM; inactivated Nav1.5, 34 μM; Kir3.1/3.4, 17 μM; Kir2.1, >>100 μM). In atrial myocytes from patients in sinus rhythm (SR) and chronic AF, XEN-D0101 inhibited non-inactivating outward currents (I_{late}) with IC_{50} of 410 and 280 nM, respectively, and peak outward currents (I_{peak}) with IC_{50} of 806 and 240 nM, respectively. Whereas I_{late} is mainly composed of I_{Kur}, I_{peak} consists of I_{Kur} and I_{to}. Therefore, the effects on I_{to} alone were estimated from a double-pulse protocol where I_{Kur} was inactivated (3.5 μM IC_{50} in SR and 1 μM in AF). Thus, inhibition of I_{peak} is because of I_{Kur} reduction and not I_{to}. XEN-D0101 significantly prolonged the atrial action potential duration at 20%, 50%, and 90% of repolarization (AF tissue only) and significantly elevated the atrial action potential plateau phase and increased contractility (SR and AF tissues) while having no effect on human ventricular action potentials. In healthy volunteers, XEN-D0101 did not significantly increase baseline- and placebo-adjusted QTc up to a maximum oral dose of 300 mg. XEN-D0101 is a Kv1.5/I_{Kur} inhibitor with an attractive atrial-selective profile.

Key Words: XEN-D0101, atrial fibrillation, Kv1.5, I_{Kur}

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INTRODUCTION

Atrial fibrillation (AF) contributes to overall cardiovascular morbidity and mortality and occurs in 1%–2% of the general population. Patients with AF have a 5-fold increased risk for stroke, and approximately one-fifth of all strokes can be attributed to AF.[1]

AF can be triggered by ectopic activity, single circuit reentry, or multiple circuit reentry.[2] Current strategies to control AF involve either sinus rhythm (SR) maintenance or heart rate control. AF termination and SR maintenance can be achieved by inhibiting K^+ channels responsible for atrial repolarization. Although SR control may be the preferred and most effective treatment of AF, none of the SR control drugs currently available are able to maintain SR without significant side effect risk.[3] Furthermore, the currently used class 3 antiarrhythmic agents lack atrial specificity, producing highly undesirable proarrhythmic liability in the ventricles that can lead to *torsade de pointes* and sudden cardiac death.[4] Thus, there is a clear unmet medical need for new pharmacological AF therapies with improved efficacy and safety.[5]

A putative atrial-selective drug target is Kv1.5, which underlies the cardiac ultrarapidly activating outward potassium current (I_{Kur}) in humans[6–9] and importantly displays atrial-specific expression.[10–12] Inhibition of I_{Kur} extends the repolarization phase of the atrial cardiac action potential in AF patients[13] to provide desirable antiarrhythmic effects without unfavorably affecting the ventricles. Selective Kv1.5/I_{Kur} inhibitors thus represent a potentially safer pharmacological intervention strategy.[14] In addition to antiarrhythmic properties, K_v1.5/I_{Kur} inhibitors may also improve atrial contractility and thereby reduce thromboembolic risk.[15]

Herein we report that XEN-D0101 alters action potentials recorded from human atrial tissue, but not human ventricular tissue, and that this is because of the selective Kv1.5/I_{Kur} modulation and possibly Kv4.3/I_{to} modulation at higher test concentrations. These observations reinforce the

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notion that selective $K_v1.5/I_{Kur}$ inhibitors represent a new class of atrial-selective antiarrhythmic agents.

METHODS

For in vitro studies, stock solutions of XEN-D0101 (a thienopyrimidine) formulated in dimethylsulfoxide (DMSO) were frozen and stored as aliquots at approximately −20°C until use. Serial dilution of stock solutions in DMSO were performed in glass vials before dilution in experimental solution to achieve the desired final perfusion concentrations, with a vehicle concentration of typically 0.1% vol/vol DMSO.

Studies reported here conform to the principles outlined in the Declaration of Helsinki were reviewed and approved by relevant Ethics Committees, and human subjects gave written informed consent. Atrial trabecular muscle from the right atrium appendage was received from SR and AF patients undergoing open heart surgery for coronary artery or valve disease (ethical no. 15.1/01031/006/2008 and EK790799). Human ventricular tissue was obtained from general organ donors whose hearts were explanted to obtain pulmonary and aortic valves for transplantation (ethical no. 339/PI/10, 4991-0/2010-1018EKU). Preparations from patients previously receiving antiarrhythmic drugs were excluded. Additional donor information is provided in **Supplemental Digital Content 3** (see **Supplementary Methods**, http://links.lww.com/JCVP/A107).

Cloned Cardiac Ion Channel Electrophysiology (Performed by Xention)

Cell lines stably expressing human Kv1.5, Kv4.3, or Nav1.5 in CHO cells and hERG, Kir3.1/3.4, or Kir2.1 in HEK293 cells were maintained in media containing 10% fetal calf serum and appropriate selection antibiotic. Cells were grown either in suspension or in T-flasks and routinely passaged. Cells for patch clamping experiments were plated onto glass cover slips before use. Cells for automated patch clamping experiments (Nav1.5) were freshly prepared on each experimental day. Standard gigaseal whole-cell patch clamp techniques were performed at room temperature using glass pipettes (2–4 MΩ). Experimental solutions are given in **Supplemental Digital Content 3** (see **Supplementary Methods**, http://links.lww.com/JCVP/A107). Patch clamp amplifier (EPC) and Pulse software from HEKA Electronics (Chester, Nova Scotia, Canada) were used. Series resistance was compensated by greater than 70%. Standard voltage protocols for Kv1.5 (V_{Hold} −80 mV, 0 mV/900 ms, −40 mV/100 ms, 0.2 Hz), Kv4.3 (V_{Hold} −80 mV, +60 mV/1 s, 0.1 Hz), Kir2.1 (V_{Hold} −60 mV, −110 mV/400 ms, 0.2 Hz), Kir3.1/3.4 (V_{Hold} −80 mV, −140 mV/100 ms, ramp +60 mV/500 ms, −80 mV, 0.1 Hz), and hERG (V_{Hold} −80 mV, +20 mV/5 s, −40 mV/5 s, 0.067 Hz) were employed. For Nav1.5, a 4-pulse voltage clamp protocol was used to elicit current after whole-cell access was achieved using a QPatch16. From a holding potential of −90 mV, an initial test pulse to −10 mV (10 ms) was applied to elicit an inward Na^+ current (P1). Further test pulses to −10 mV (all 10 ms duration) were applied after conditioning steps to either −90 mV (P2) for 500 ms to relieve channel inactivation or 5 seconds to −70 mV to promote inactivation (P3). Finally, the membrane was repolarized back to −90 mV for 100 ms before a final test pulse to −10 mV was delivered to measure recovery from inactivated block (P4). The command voltage was applied every 15 seconds. After a stabilization period, a cumulative concentration response experiment was performed. Peak amplitude of the inward Nav1.5 current of P2 and P3 was measured at the end of each drug application period.

Isolation of Myocytes and Recording of I_{peak} and I_{late} Outward Currents From SR and AF Human Atrial Tissue at 37°C (Performed by Dresden University of Technology)

See **Supplemental Digital Content 3** (see **Supplementary Methods**, http://links.lww.com/JCVP/A107) for atrial myocyte isolation procedures. Experiments were performed at physiological temperature (37°C). Myocytes held at −60 mV were subject to a double-pulse protocol (0.2 Hz) to +50 mV (500 ms each) separated by an intermediate 25 ms step to −60 mV. Peak outward currents (I_{peak}) of the first test pulse were analyzed to determine mixed effects on I_{to} and I_{Kur}. Late outward current mainly represented effects on slowly inactivating I_{Kur}. The area under the curve of the initial 50 ms of the second clamp step is a measure of charge transfer and was analyzed as indicator of I_{to}.

In Vitro Human Action Potential Studies at 37°C (Performed by Dresden University of Technology and University of Szeged)

Tissue preparations were stimulated at 1 Hz with a 2-ms square wave stimulus. Measured parameters were resting membrane potential (RMP), action potential amplitude (APA), action potential duration at 20%, 50%, 75%, and 90% repolarization (APD_{20}, APD_{50}, APD_{75}, and APD_{90}), maximum depolarization rate, dV/dt_{max}, conduction time (ct), and "plateau potential" (PLT) defined as the mean membrane potential between 20% and 30% of APD (PLT_{20}).

In Vitro Human Atrial Contractility Studies (Performed by Dresden University of Technology)

The study included 14 preparations from 8 patients in SR group, and 9 preparations came from 5 patients with chronic AF. Preparations had a mean length 4.6 ± 0.3 mm and a diameter of 1.4 ± 0.1 mm (n = 23). Resting tension of each preparation was adjusted to yield half maximum active force development. The trabeculae were mounted in an organ bath that contained 50 mL of oxygenated Tyrodes solution circulating via the gas stream. The composition of the bath solution was 126.7 mM NaCl, 0.42 mM NaH_2PO_4, 22 mM $NaHCO_3$, 5.4 mM KCl, 1.8 mM $CaCl_2$, and 1.5 mM $MgCl_2$, pH 7.4, when equilibrated with 5% CO_2 in O_2 at 37 ± 0.3°C. The preparations were stimulated regularly at a frequency of 1 Hz. After a stabilization period of 60–90 minutes, XEN-D0101 was added in cumulatively increasing concentrations allowing at least 15 minutes for the effect to develop before the next increment in concentration. At the end of each

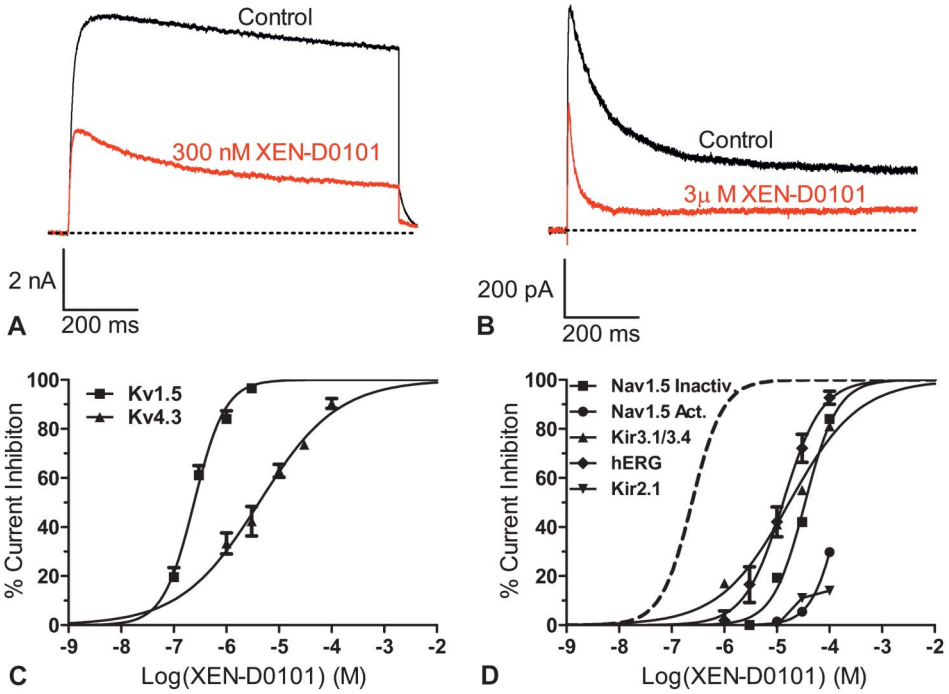

FIGURE 1. Effect of a range of concentrations of XEN-D0101 on cloned cardiac ion channel expressed in mammalian cells. A, Representative steady state Kv1.5 current traces in control and after exposure to XEN-D0101. Current inhibition at the end of the depolarizing step to 0 mV was 77% for this cell. B, Kv4.3 current traces in the absence and presence of 3 μM XEN-D0101. Peak Kv4.3 current inhibition for the cell shown was 43.7%. C, Mean Kv1.5 and Kv4.3 current inhibition (±SEM) data are shown plotted as a function of XEN-D0101 concentration and fitted with a sigmoidal function (constrained at 0% and 100%) yielding a Kv1.5 IC_{50} of 241 nM [95% confidence interval (CI), 152–383 nM, n = 6–12 cells at each concentration] and a Hill coefficient (n_H) of 1.3 ± 0.2 and a Kv4.3 IC_{50} of 4.2 μM (95% CI, 2.8–6.4 μM, n = 5 cells at each concentration, $P < 0.0001$, 2-tailed *t* test, cf. Kv1.5) and n_H of 0.6 ± 0.1. D, Mean current inhibition by XEN-D0101 is plotted as a function of concentration and fitted with a sigmoidal function (constrained at 0% and 100%) to yield a hERG IC_{50} of 13 μM (95% CI, 11.3–15.1 μM, n = 4–6 cells at each concentration, $P < 0.0001$, 2-tailed *t* test, cf. Kv1.5) and an n_H of 1.3 ± 0.2. Effect of XEN-D0101 on Nav1.5 current was evaluated on the QPatch16 using a 4-pulse protocol described in the Methods. Mean Nav1.5 current inhibition data are shown plotted as a function of XEN-D0101 concentration yielding IC_{50} values for Peak 3 (Nav1.5 inactivated-state block): 34.4 μM (95% CI, 21.9–53.9 μM, $P < 0.0001$, 2-tailed *t* test, cf. Kv1.5) and n_H of 1.4 ± 0.2 and Peak 2 (Nav1.5 activated-state block): >100 μM (n = 6 cells). Mean inhibition data for Kir3.1/3.4 are shown plotted as a function of XEN-D0101 concentration yielding IC_{50} value of 17 μM (95% CI, 12–24 μM, n = 4–6 cells, $P < 0.0001$, 2-tailed *t* test, cf. Kv1.5) and an n_H of 0.7 ± 0.1. Mean inhibition data for Kir2.1 are shown plotted as a function of XEN-D0101, nominal inhibition of current was observed over a range of concentrations up to 100 μM, and no meaningful fit to these data was possible. Kv1.5 fit data are shown as a dashed line for comparison.

experiment, the preparations were exposed to 8 mM $CaCl_2$ for assessment of maximum force development.

QTc Evaluation in Healthy Volunteers (Performed by Xention)

A double-blind, randomized, placebo-controlled phase 1 study was performed to determine safety and tolerability of XEN-D0101 and to carefully examine the drug plasma concentration–QTc relationship. Healthy male volunteers were recruited into the study, and volunteers were specifically excluded if they had a clinically significant electrocardiographic (ECG) abnormality, cardiac arrhythmia, heart disease, hypokalemia, hyperkalemia, structural heart disease, bradycardia, myocardial channelopathy, or mean QTc (Fridericia correction) greater than 450 ms. Subjects remained resident in the Clinical Pharmacology Unit throughout the study period. Six groups of 8 subjects (fasted overnight) took a single dose of XEN-D0101 or placebo. At each dose level, 6 subjects were randomized to receive active treatment and 2 subjects received placebo. Dosages investigated were 15, 30, 45, 75, 150, and 300 mg XEN-D0101. Blood samples for assay of XEN-D0101 were taken before and frequently up to 48 hours after each single dose. Assay of plasma concentrations of XEN-D0101 was performed using a high-performance liquid chromatography, tandem mass spectroscopy method. The lower limit of quantification for the assay was 0.5 ng/mL. Safety ECG telemetry (1-lead ECG) was monitored continuously from dosing until 24 hours afterward, using a SpaceLabs system to monitor heart function and detect proarrhythmic events in real time. To accurately detect drug-related changes in the QTc interval, continuous 12-lead ECGs were recorded using SEER MC recorders (GE Healthcare) with Mason–Likar electrodes. A continuous 12-lead ECG was recorded for 14 hours, starting at 1 hour before dosing and finishing at 13 hours after dosing (and at corresponding times on Day 1). Recordings were also made on Day 2 (23−37 hours after dosing). ECG analysis was performed on approximately 30,000 ECGs using a semiautomatic method with sufficient precision and reproducibility as described elsewhere.[16] As ECGs were measured automatically, 20% of the measured ECGs were selected (either randomly or targeting the extreme QT- and RR-interval (QT, RR) measurements of each participant) and reviewed by an experienced and medically qualified observer. The QT interval was corrected for heart rate using individual regression equations. In each subject, the regression model that provided

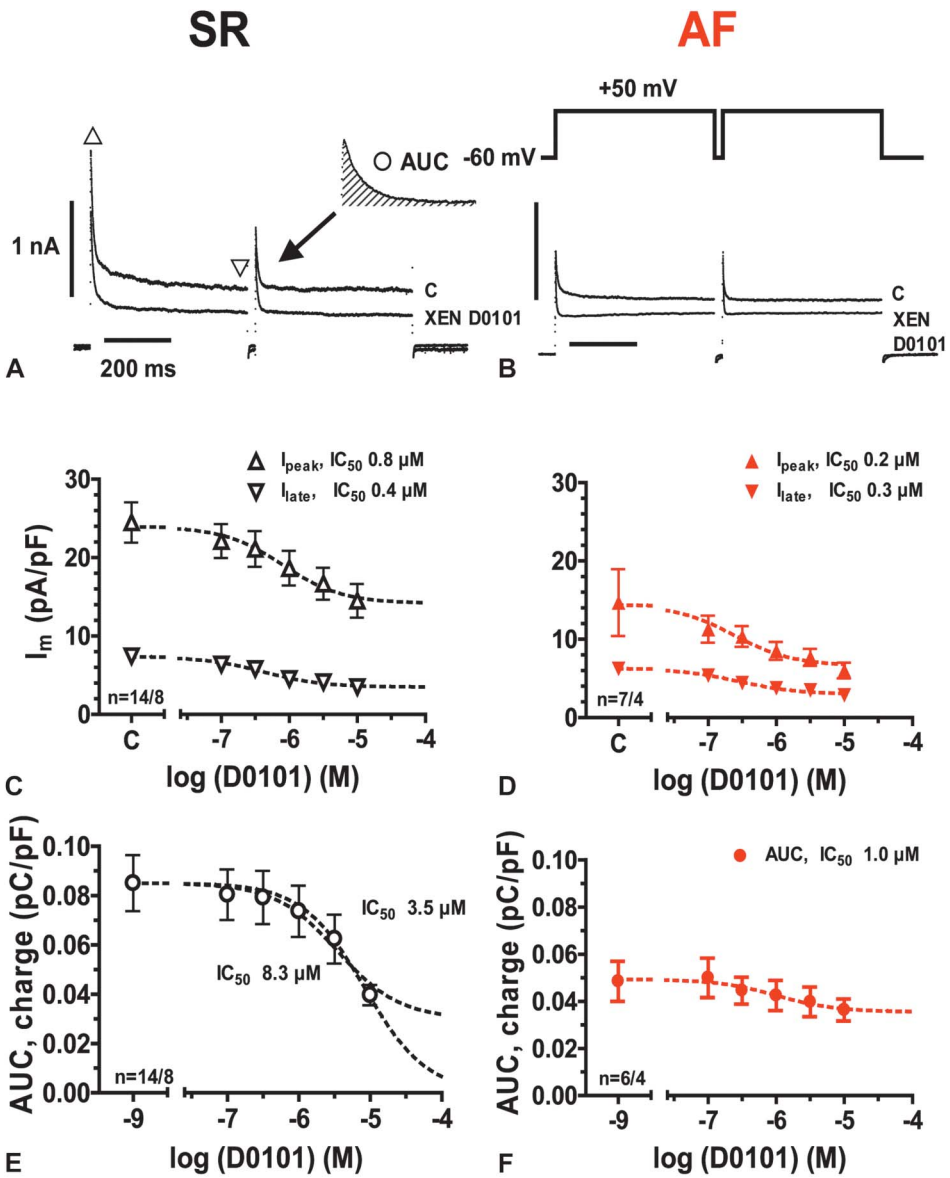

FIGURE 2. Effect of a range of concentrations of XEN-D0101 on I_{peak}, I_{late}, and I_{to} currents recorded from human atrial myocytes from SR and AF donors at 37°C. A and B, Original current recordings, control and in the presence of 10 µM XEN-D0101, from SR and AF are shown. Double-pulse protocol—holding potential: −60 mV, 2 voltage steps to +50 mV, duration 500 ms, intermediate pulse to −60 mV, duration 25 ms (see inset in B). Effects on I_{to} were quantified by the area under the curve (AUC) of the inactivating current of the second pulse (inset in A). C and D, Concentration-dependent effects of XEN-D0101 on peak current (I_{peak}) and late current (I_{late}) in SR and AF are shown. Dashed lines represent adapted Hill equations assuming a Hill slope of 1 to the mean data with IC_{50} values for SR of 806 nM [562–1152 nM, 95% confidence interval (CI)] and 413 nM (308–554 nM, 95% CI) for I_{peak} and I_{late}, respectively, and for AF 241 nM (136–429 nM, 95% CI) and 281 nM (112–708 nM, 95% CI) for I_{peak} and I_{late}, respectively. E and F, Concentration-dependent effects of XEN-D0101 on I_{to} measured as AUC (in pC/pF) of inactivating current component during the initial 50 ms of second voltage step (see inset in A) for SR and AF are shown. Two alternative curve adaptations were conducted assuming the maximum value = mean control, Hill coefficient = 1, and either (1) a minimum value of 0.03 pC/pF or (2) full block (0 pC/pF). The respective IC_{50} values were 3.5 µM (2.4–5.1 µM, CI) and 8.3 µM (4.3–16.2 µM, CI). For AF, the IC_{50} was 1.0 µM (0.56–1.9 µM, CI). n = numbers (cells per patients). Mean data ± SEM.

the lowest regression residuals was converted into an individualized heart rate correction, which yielded a QT corrected for heart rate (QTc). The means of the repeated measurements on Day 1 were used to derive time-matched changes from baseline (ΔQTc). The subjects who received placebo in individual cohorts were pooled (n = 12), and their time-matched ΔQTc readings on days 1 and 2 were averaged at each time point to obtain a ΔQTc placebo profile of the study. The on-treatment ΔQTc profiles were corrected for placebo to obtain ΔΔQTc profiles for each treatment group.

Statistical Analysis

Data are typically presented as mean ± SEM, and where appropriate, 95% confidence intervals are given. Student *t* test or 1-way analysis of variance (ANOVA) with multiple measurements were used as appropriate. Differences were considered statistically significant for $*P < 0.05$, $§P < 0.01$, and $\#P < 0.001$. Concentration effect modeling was performed for QTc evaluation in healthy volunteers with ΔΔQTc intervals being regressed onto the plasma concentrations of XEN-D0101 at the exact times when the continuous 12-lead ECGs were recorded. The effect of XEN-D0101 plasma concentration on ΔΔQTc was investigated using a regression model and ANOVA. At each time of stable supine rest, XEN-D0101 plasma concentration was interpolated using nonparametric superposition (see **Figure 1, Supplemental Digital Content 1**, http://links.lww.com/JCVP/A105). A mixed-effects model, which included plasma concentration,

treatment group, and interaction between plasma concentration and treatment group, as fixed factors, and subject as a random factor, was evaluated.

RESULTS

Effect of XEN-D0101 on Cloned Cardiac Ionic Channels

The pharmacological profile of XEN-D0101 against several ion channels known to be expressed and functionally important in the human heart is reported in Figure 1 (original current traces are shown in **Figure 2, Supplemental Digital Content 2**, http://links.lww.com/JCVP/A106). XEN-D0101 inhibited Kv1.5 (241 nM IC_{50}) and was shown to be 17-fold selective over Kv4.3, greater than 50-fold selective over hERG, greater than 70-fold selective over Kir3.1/3.4, and greater than 100-fold selective over Nav1.5 and Kir2.1. Although only observed at very high test concentrations, XEN-D0101 preferentially blocked inactivated-Nav1.5 over open-Nav1.5 channels.

Effect of XEN-D0101 on I_{peak} and I_{late} Currents Recorded From Human Atrial Myocytes

At physiological temperature, XEN-D0101's ability to inhibit I_{peak} (peak outward currents = I_{to} and I_{Kur}) and I_{late} (late outward currents = slowly inactivating I_{Kur} and other uncharacterized currents, also called I_{sus} in previous studies[6]) was determined in human atrial myocytes from SR and AF donors. Additionally, the charge carried during the fast inactivating current component of the second voltage step (Fig. 2A, inset) was used as surrogate to estimate the effect on I_{to} (mainly conducted by Kv4.3). XEN-D0101 concentration dependently reduced all current components, although with different sub-micromolar potencies in both SR and AF atrial myocytes (Fig. 2). In SR, the IC_{50} for I_{peak} was approximately 4-fold greater than the corresponding value in AF. Inhibition of I_{late}, largely representing noninactivating I_{Kur}, is similar in SR and AF tissues (Figs. 2C, D). Inhibition of I_{to} based on area under the curve/charge of the inactivating peak current during the second voltage step is pronounced in SR but small and perhaps not biologically relevant in AF because of reduced contributions of I_{to} in AF (Figs. 2E, F). The IC_{50} selectivity ratio of I_{late} over I_{to} in SR tissue calculates between 8- and 20-fold depending on the assumption for IC_{50} determination (Fig. 2E).

Effect of XEN-D0101 on Human Cardiac Action Potentials

Effects on human atrial and ventricular action potentials are shown in Figure 3 and Table 1. The most striking concentration-dependent effects of 1 and 3 μM XEN-D0101 in AF atrial trabeculae were robust elevation in plateau potential (>16 mV) and prolongation in APD_{20} (>47 ms), APD_{50} (>47 ms), and APD_{90} (>30 ms). In SR atrial trabeculae, XEN-D0101 also significantly elevated the plateau potential (>26 mV) but in contrast to AF preparations significantly reduced APD_{90} (<84 ms). Resting potential became significantly less negative (by 3 mV) in SR atrial tissue. All effects were at least partially reversible upon drug washout with the exception of APD_{90} shortening in SR tissue. Action potential amplitude, dV/dt_{max}, and conduction time were not altered. XEN-D0101 up to 3 μM did not significantly alter any action potential parameter in human ventricular tissue. Consistent with what has been reported elsewhere in the literature under control conditions, the resting membrane potential of SR and AF atrial cells was more depolarized than observed in ventricular cells and SR atrial cells were more depolarized than AF atrial cells.[17]

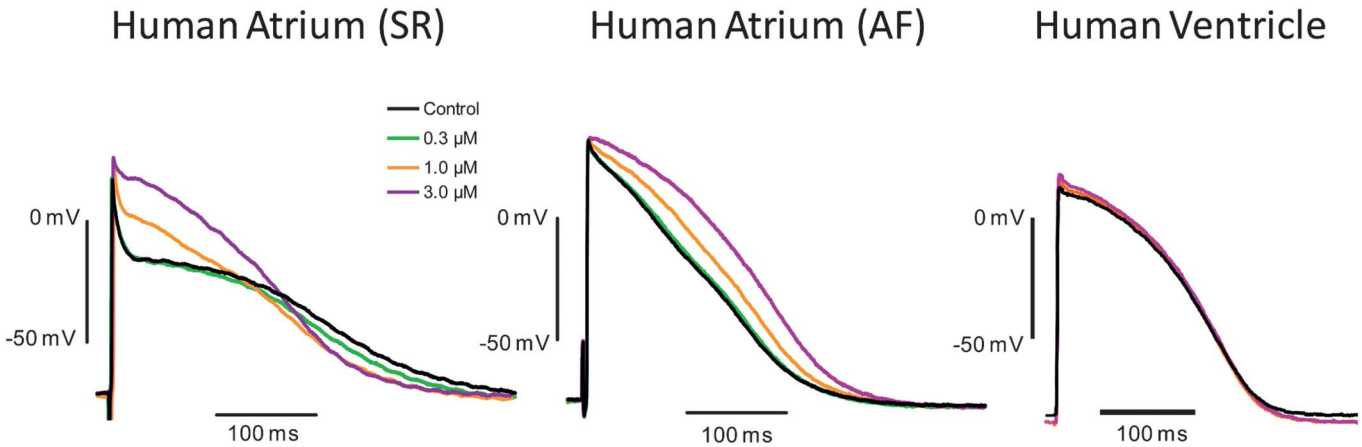

FIGURE 3. Effects of XEN-D0101 on cardiac action potentials. Example of individual tracings recorded in the presence of increasing concentrations of XEN-D0101 shown at the end of the exposure period of 20 minutes. The effects of XEN-D0101 on human action potentials were examined in right atrial trabeculae from patients in SR and AF and human ventricle at 1 Hz. The compound concentration dependently elevated the plateau phase of the action potential in atrial tissue only. In SR, this effect was associated with shortening of APD_{90}, whereas prolongation of APD_{90} was observed in AF. XEN-D0101 did not change action potential parameters recorded from human ventricular tissue. AP measurements and number (n) are provided in Table 1.

TABLE 1. Effect of XEN-D0101 on Action Potentials Recorded From Human Atrial and Ventricular Tissues at 1 Hz

Effect of XEN-D0101 on action potentials recorded from AF human atrial trabecular muscle (n = 6)

Parameter	Control	0.3 μM	1 μM	3 μM	Wash
RMP (mV)	−77 ± 1§	−77 ± 1	−77 ± 1	−76 ± 1	−77 ± 0
APA (mV)	100 ± 3§	102 ± 3	102 ± 3	103 ± 3	102 ± 3
APD_{20} (ms)	40 ± 8§	40 ± 8	59 ± 7#	87 ± 5#	32 ± 10
APD_{50} (ms)	106 ± 9*	108 ± 9	127 ± 8§	153 ± 6#	108 ± 12
APD_{90} (ms)	202 ± 8#	204 ± 8	213 ± 9	232 ± 8#	210 ± 12
dV/dt_{max} (V/s)	180 ± 27	181 ± 37	181 ± 45	175 ± 45	173 ± 36
ct (ms)	5 ± 1	5 ± 1	5 ± 1	5 ± 1	5 ± 1
PLT_{20} (mV)	2 ± 3§	3 ± 3	11 ± 3§	18 ± 2#	−3 ± 4

Effect of XEN-D0101 on action potentials recorded from SR human atrial trabecular muscle (n = 6)

Parameter	Control	0.3 μM	1 μM	3 μM	Wash
RMP (mV)	−72 ± 1	−71 ± 1	−71 ± 1	−69 ± 1*	−69 ± 1
APA (mV)	91 ± 1	93 ± 2	93 ± 2	95 ± 2	91 ± 2
APD_{20} (ms)	6 ± 0	6 ± 1	10 ± 2	58 ± 16§	6 ± 1
APD_{50} (ms)	153 ± 13	148 ± 11	134 ± 14	148 ± 13	107 ± 25
APD_{90} (ms)	337 ± 12	319 ± 16	282 ± 17*	253 ± 14#	258 ± 29#
dV/dt_{max} (V/s)	145 ± 10	156 ± 15	149 ± 6	145 ± 4	137 ± 7
ct (ms)	4 ± 0	4 ± 0	4 ± 0	4 ± 0	4 ± 0
PLT_{20} (mV)	−16 ± 2	−14 ± 2	−10 ± 2	10 ± 5#	−18 ± 3

Effect of XEN-D0101 on action potentials recorded from human ventricular tissue (n = 6–8)

	Control	1 μM	Control	3 μM
RMP (mV)	−87 ± 1	−88 ± 1	−87 ± 1	−86 ± 1
APA (mV)	106 ± 2	108 ± 2	108 ± 3	110 ± 2
APD_{25} (ms)	147 ± 10	145 ± 10	131 ± 15	140 ± 15
APD_{50} (ms)	208 ± 15	210 ± 16	188 ± 13	198 ± 16
APD_{90} (ms)	273 ± 21	277 ± 23	249 ± 12	257 ± 14
dV/dt_{max} (V/s)	249 ± 36	245 ± 64	292 ± 49	255 ± 33

XEN-D0101 concentration dependently elevated the plateau phase of the action potential in SR and AF atrial trabecular muscle. In SR, this effect was associated with shortening of APD_{90}, whereas prolongation of APD_{50} and APD_{90} was observed in AF. XEN-D0101 significantly increased the RMP by 3 mV in SR atrial trabecular muscle. Multiple measures ANOVA with Bonferroni postcomparison test was used for statistical analysis of drug effects versus control values for each AP parameter. Student *t* test was used to compare control action potential parameters in SR and AF.

*$P < 0.05$, §$P < 0.01$, #$P < 0.001$.

APA, action potential amplitude; ct, conduction time.

Effect of XEN-D0101 on Human Atrial Tissue Contractility

In human SR atrial tissue, XEN-D0101 significantly increased contractility at 3 μM (>30%) and 10 μM (>38%) but only at 10 μM (>63%) in human AF atrial tissue. The force of contraction was greater in the presence of both XEN-D0101 and 3 mM 4-action potential compared with XEN-D0101 alone, but this did not match the maximum force of contraction observed in the presence of 8 mM Ca^{2+}. These results are reported in Figure 4.

Effect of XEN-D0101 on QTc Interval and Drug-induced Proarrhythmia in Healthy Human Subjects

Comprehensive ECG recordings and ECG analysis were performed in a phase 1 clinical trial to determine whether XEN-D0101 prolongs the QTc interval in human. The quality of the ECG data from this study was high with 90% of subjects having a regression residual below 6 ms. XEN-D0101 plasma concentration plotted against ΔΔQTc is given in Figure 5, and most notably, XEN-D0101 plasma concentrations exceeded 3000 ng/mL (~10 μM drug concentration), in some volunteers without increasing ΔΔQTc. The regression coefficients for plasma concentration versus ΔΔQTc derived from the mixed-effects model were not significantly different from zero ($P = 0.1990$). The ANOVA analysis of the individual model components showed that XEN-D0101 plasma concentration did not significantly affect ΔΔQTc ($P = 0.6719$). Categorical analysis of QT and QTc was performed, and no subjects on active treatment had a QT or QTc greater than 450 ms or increase in QTc from baseline greater than 30 ms. In addition to 12-lead Holter monitoring, single-lead ECG telemetry monitoring was performed continually throughout the clinical trial. All results of ECG telemetry were considered by the investigator to be normal. There were no clinically significant changes, including induction of AF or ventricular proarrhythmia after XEN-D0101 dosing.

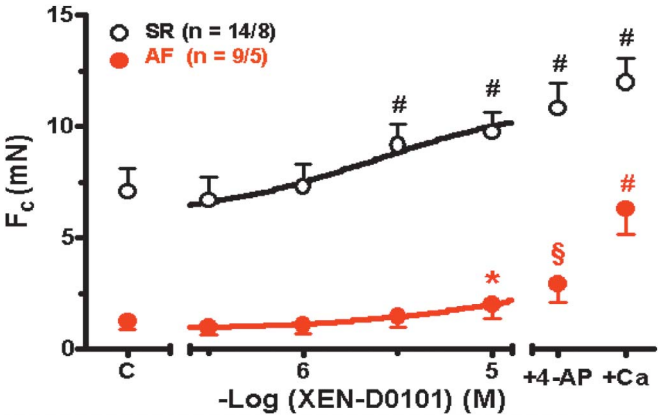

FIGURE 4. Effects of XEN-D0101 on atrial tissue contractility. Effects of XEN-D0101 and 3 mM 4-AP in addition to XEN-D0101 on force of contraction of isolated human right atrial trabeculae from patients in SR (black symbols) and AF (red symbols). At the end of each experiment, 8 mM $CaCl_2$ (+Ca) was added for the assessment of maximum force of contraction. C, pre-drug control. n = Number of trabeculae/number of patients. *$P < 0.05$, §$P < 0.01$, #$P < 0.001$ versus pre-drug control.

DISCUSSION

Ion channel electrophysiology studies performed against native and cloned cardiac ion channels demonstrated that XEN-D0101 preferentially targets Kv1.5/I_{late} (ie, I_{Kur}) with moderate selectivity over Kv4.3/I_{to} (between 8- and 20-fold). XEN-D0101 is selective for Kv1.5 over hERG (>50-fold), Kir3.1/3.4 (>70-fold), and Nav1.5 and Kir2.1 (>100-fold). Inhibition of I_{late} was demonstrated in both SR and AF atrial tissues, whereas I_{to} was significantly downregulated in AF atrial tissue and therefore difficult to measure. Comparing recent studies on I_{Kur} remodeling in AF, downregulation of I_{Kur} is less pronounced than that of I_{to}.[13] Also in this investigation, the late component of outward current is not significantly different from SR. There are indications that besides I_{Kur}, other currents may contribute to the late current.[13]

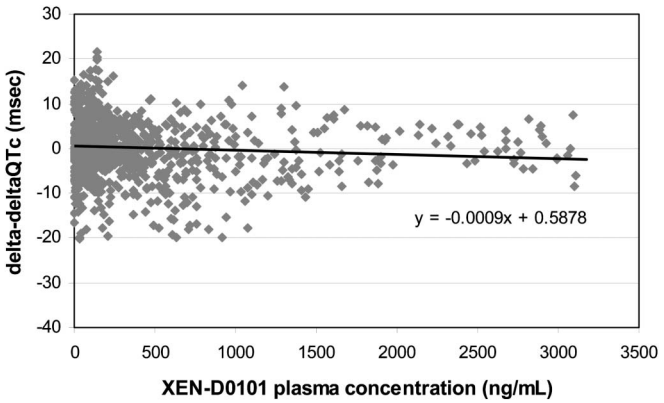

FIGURE 5. XEN-D0101 plasma concentration plotted against baseline- and placebo-adjusted QTc (ΔΔQTc) values derived from healthy volunteers (n = 63). Concentration effect modeling showed no evidence of a consistent relationship between plasma concentration of XEN-D0101 and ΔΔQTc in healthy volunteers orally dosed with XEN-D0101.

Findings reported here for human cardiac action potential studies are consistent with the ion channel pharmacology of XEN-D0101. Action potential recordings were performed with atrial tissue from patients who either were in SR or had a history of chronic AF for at least 6 months duration. Action potentials from patients in SR showed a characteristic spike and dome configuration, whereas those from patients in AF had a triangulated shape.[18] The triangular shape of action potentials from AF preparations is the result of electrical remodeling driven by altered ion channel expression and regulation.[19] In this study, XEN-D0101 evoked changes similar to previously reported effects of 4-AP, which at concentrations selective for I_{Kur} block elevated the plateau potential in SR and AF preparations and significantly shortened APD_{90} in SR but prolonged APD_{90} in AF trabecula.[18] However, in the presence of XEN-D0101, the reduction in APD_{90} in SR tissue was not reversible in the washout period, unlike plateau elevation in SR and AF tissues and APD_{90} increase in AF tissue, suggesting that this effect may not be drug related. A possible explanation for this is that Ca^{2+} current rundown is occurring in SR tissue, and this effect is absent or much smaller in AF tissue because I_{Ca} is already downregulated. The concentrations of XEN-D0101 required to change action potential parameters were 1 and 3 μM, which is higher than the Kv1.5/I_{late} IC_{50} value. The reason for this difference could relate to drug accessibility to the Kv1.5 channels being much greater in single dissociated myocytes compared with large multicellular preparations, perhaps because of permeability/diffusion problems. Bioanalysis to measure XEN-D0101 levels in cardiac tissue was not performed to confirm this hypothesis. Additionally, because the action potential is a multichannel event, the effect that a conductance change in a single-channel type has on the overall shape of the action potential depends on the relative contribution to the momentary overall sum of conductances. Similar differences in potencies on current and action potential parameters are regularly observed. Data from recombinant Kir2.1 and Kir3.1/3.4 channels suggest that the small depolarization in resting membrane potential observed in SR tissue, which is unlikely to be biologically relevant, is not because of inhibition of native I_{K1} or I_{KACh}.

XEN-D0101 increased contractile force in human SR and AF atrial trabeculae in a concentration-dependent manner. Similarly, elevation of the plateau phase of the human atrial action potential by 4-AP or via the nonselective I_{Kur} and I_{to} blocker AVE0118 leads to a concentration-dependent positive inotropic effect.[15,18] The increase in force of contraction after elevation of the plateau phase has been explained by an indirect enhancement of Ca^{2+} influx via voltage-dependent L-type Ca^{2+} channels[18] or via Na^{+}–Ca^{2+} exchanger operating in its reverse mode.[20] AF is associated with marked contractile dysfunction that can be partially counteracted by 4-AP; however, as demonstrated here, the positive inotropic effect is less robust than in SR. The positive inotropic effects of XEN-D0101 at 10 μM are unlikely to be because of only Kv1.5/I_{late} inhibition due to lack of positive inotropic effects at 1 μM,

which is known to significantly alter atrial action potential parameters and selectively inhibit Kv1.5/I_{late}.

The absence of XEN-D0101–induced QTc prolongation and proarrhythmia in healthy volunteers in the clinical study referenced herein is an important safety milestone that has not previously been reported for selective Kv1.5 drugs. Indeed, it has previously been reported that a "loss of function" KCNA5 polymorphism may induce susceptibility to AF in human, although in vivo causality was not demonstrated in this study.[21] The ability of XEN-D0101 to induce AF was carefully evaluated in this clinical trial with continuous ECG monitoring, and no incidents of atrial tachycardia or AF were reported. The lack of drug-induced QTc prolongation in healthy volunteers is consistent with the human ventricular action potential recordings performed in the presence of XEN-D0101. However, it remains to be determined whether the same safety conclusions can be made with respect to the AF patient population, who often experience electrical and structural remodeling in the heart because of cardiovascular disease and ageing. The total drug plasma concentrations in some of the healthy volunteers in the phase 1 clinical study reached ~10 μM (1 μM unbound drug concentration), a concentration that maximally modulates Kv1.5.

CONCLUSIONS

Studies reported herein demonstrate that XEN-D0101 is selective for Kv1.5/I_{late} over nontarget cardiac ion channels and in accordance with these pharmacological properties selectively influences various action potential and contractility parameters in human atrial AF tissue but not human ventricular tissue. It remains to be demonstrated that these atrial ex vivo findings translate to atrial-selective antiarrhythmic properties in AF patients.

STUDY LIMITATIONS

It has to be admitted that the number of tissue samples from patients is relatively low. The variability of electrophysiological parameters in human ex vivo preparations is on the other hand large. Because AF preparations contract much less than SR preparations, action potentials are generally more stable in the AF group. Studying Ca^{2+} handling is beyond the scope of the study; however, effects on force of contraction were added. We interpret that the increase in force of contraction with the profound elevation of plateau phase by XEN-D0101, especially in SR which indirectly enhances Ca^{2+} entry,[18] and an inhibitory effect on Ca^{2+} release seems unlikely.

Although a very high number of ECGs were analyzed in this XEN-D0101 clinical study to provide excellent assay sensitivity for detecting drug-related changes in the QTc interval, this particular study was not performed according to ICH E14 regulatory guidance. In particular, a positive pharmacological control known to prolong the heart rate–corrected QTc interval to an expected level was not included and ECG monitoring covering the pharmacokinetic profile of both the parent compound and the metabolites was not performed.

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