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BASIC SCIENCE

Is the acetylcholine-regulated inwardly rectifying potassium current a viable antiarrhythmic target? Translational discrepancies of AZD2927 and A7071 in dogs and humans

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Aims We aimed at examining the acetylcholine-dependent inward-rectifier current (I_{KACh}) as a target for the management of atrial fibrillation (AF).

Methods and results The investigative agents AZD2927 and A7071 concentration-dependently blocked I_{KACh} *in vitro* with minimal off-target activity. In anaesthetized dogs ($n = 17$) subjected to 8 weeks of rapid atrial pacing (RAP), the left atrial effective refractory period (LAERP) was maximally increased by 50 ± 7.4 and 50 ± 4.8 ms following infusion of AZD2927 and A7071. Ventricular refractoriness and the QT interval were unaltered. During sustained AF, both drugs significantly reduced AF frequency and effectively restored sinus rhythm. AZD2927 successfully restored sinus rhythm at 10/10 conversion attempts and A7071 at 14/14 attempts, whereas saline converted 4/17 episodes only ($P<0.001$ vs. AZD2927 and A7071). In atrial flutter patients ($n = 18$) undergoing an invasive investigation, AZD2927 did not change LAERP, the paced QT interval, or ventricular refractoriness when compared with placebo. To address the discrepancy on LAERP by I_{KACh} blockade in man and dog and the hypothesis that atrial electrical remodelling is a prerequisite for I_{KACh} blockade being efficient, six dogs were studied after 8 weeks of RAP followed by sinus rhythm for 4 weeks to reverse electrical remodelling. In these dogs, both AZD2927 and A7071 were as effective in increasing LAERP as in the dogs studied immediately after the 8-week RAP period.

Conclusion Based on the present series of experiments, an important role of I_{KACh} in human atrial electrophysiology, as well as its potential as a viable target for effective management of AF, may be questioned.

Keywords Antiarrhythmic agents • Arrhythmia • Electrophysiology • Ion channels • Remodelling

Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia encountered in clinical practice and contributes markedly to population morbidity and mortality conferring a five-fold increased risk of stroke and a doubled mortality rate independently of other known predictors of death.[1] The disease is progressive and is associated with heart failure, frequent hospitalizations, poor quality of life, and significant socioeconomic burden.[1] Management of AF patients aims at reducing symptoms and preventing severe complications, therapeutic goals that need to be pursued in parallel. Current treatment guidelines recommend initiation of AF therapy with safer, although less efficacious, antiarrhythmic agents with attention paid to underlying structural heart disease.[1] Presently available agents possess limited efficacy and significant risks underpinning the need for novel strategies to restore and maintain normal sinus rhythm.[1–3]

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What's new?

- AZD2927 and A7071 are two novel agents selectively blocking the acetylcholine-dependent inward-rectifier current (I_{KACh}) *in vitro*.
- In both naive dogs and dogs subjected to extended periods of rapid atrial pacing, I_{KACh} blockade is associated with a pronounced increase in atrial refractoriness indicating a role of constitutively active Kir3 channels in both cases.
- In patients with a history of atrial flutter, selective I_{KACh} blockade by AZD2927 does not result in an increased atrial refractoriness.

An attractive prospect for improved AF therapy would be drugs that target ion channels specifically contributing to atrial repolarization, thus avoiding ventricular proarrhythmia liability.[2,3] Strategies selectively blocking atrially expressed K^+-channels like the acetylcholine-dependent inward-rectifier current (I_{KACh}) and the ultrarapid delayed-rectifier current (I_{Kur}) are currently under clinical scrutiny.[2,4–6] The K_{ACh} channel is a tetrameric complex of two Kir3.1 and two Kir3.4 subunits and is activated by acetylcholine binding to the muscarinic M2 receptor.[3] An increased activity of inward I_{KACh} abbreviates atrial APD and promotes AF through wavelength shortening. Since the pore-forming Kir3 alpha subunits are predominantly expressed in atria, block of I_{KACh} has been suggested as an interesting approach for AF treatment.[3,4] Clinical observations indicate that vagal activity plays an important role in the pathogenesis of AF as many patients experience the onset of AF episodes during sleep, after meals, and at other occasions when vagal tone is high which indicate that I_{KACh} might be involved in triggering and maintaining AF.[7] Furthermore, as I_{KACh} seems constitutively active in patients with long-lasting AF, block of I_{KACh} may be a promising option not only for the treatment of vagally dependent AF but also for AF in general.[3,8] Support for this idea stems from studies in which the selective Kir3 blocker tertiapin-Q showed antiarrhythmic efficacy in dogs and in rat atrial cardiomyocyte cultures and intact atria subjected to atrial tachypacing.[9,10] Atrial specificity was evidenced by the absence of ventricular cardiomyocyte currents or APD prolongation and relative tertiapin-Q-induced action potential prolongation was significantly larger in electrically remodelled atrial tissue isolated from atrially tachypaced dogs than in tissue from control dogs. However, as for the strategy of blocking I_{Kur}, the value of selectively targeting I_{KACh} still awaits clinical proof-of-concept testing in an atrial arrhythmia population.

AZD2927 ((*S*)-4-fluoro-*N*-(1-(3-hydroxyazetidin-1-yl)-3-methylbutan-2-yl)-*N*,3-dimethylbenzamide), and A7071 ((*S*)-4-cyano-*N*-(2-(3-hydroxyazetidin-1-yl)-1-phenylethyl)-*N*-methylbenzamide) is the result of efforts in identifying novel antiarrhythmic agents that selectively block I_{KACh}. The major objectives of the present series of experiments were to characterize their ion channel-blocking potencies *in vitro* and their electrophysiological and antiarrhythmic effects in dogs subjected to 8 weeks of rapid atrial pacing (RAP) to induce atrial electrical remodelling and fibrillation. Based on its electrophysiological characteristics and safety profile, AZD2927 was forwarded to clinical testing in atrial flutter patients to assess its safety and tolerability, pharmacokinetic properties, and electrophysiological effects.

Methods

Preclinical studies

These experiments apply to the European Commission guidelines and were approved by the local ethics committee on animal experiments in Gothenburg, Sweden [applications 101087 (281–2009) and 101443 (45–2012)].

Ion channel-blocking effects *in vitro*

The ion channel-blocking potency of AZD2927 and A7071 was assessed in Chinese Hamster Ovary (CHO) cells stably expressing the human cardiac ion channels hKir3.1/hKir3.4/hM2, hERG, hKvLQT1/hminK, hKv1.5, hKv4.3/hKChIP2.2, hCav1.2, or hNav1.5. Electrophysiological studies on recombinant ion channels were performed using a high-throughput planar patch clamp assay. The blocking potency of AZD2927 and A7071 on I_{KACh} was also assessed using the whole-cell variant of the patch clamp method in human atrial cardiomyocytes isolated from atrial tissue excised during cardiac surgery Detailed methodological descriptions of the *in vitro* preclinical studies can be found in the Supplementary material online, supplementary file.

Antiarrhythmic efficacy assessment of AZD2927 and A7071 during 8 weeks of rapid atrial pacing in dogs

To attenuate potential negative impact on ventricular function by the RAP and to avoid atrio-ventricular ablation, dogs with slow ventricular response rates to the RAP were screened. Beagle dogs of both genders were sedated with acepromazin (0.15 mg/kg) and anaesthetized with propofol (6 mg/kg) and isoflurane (1.5–2.5%). Subsequently, an oesophageal-pacing electrode (25125 ESOFLEX 2 PU 2, FIAB SpA) was introduced to pace the atria at 400 beats/min. Ventricular rate was monitored using a pulse oximeter (Ohmeda® 4700 OxiCap, Ohmeda) and dogs with a ventricular response rate of $<$125 beats/min were selected for pacemaker implantation. Seventeen dogs (11.7–17.8 kg body weight) considered suitable for RAP were sedated and anaesthetized as described above with the addition of buprenorphine (0.015 mg/kg). A neurostimulator (Itrel®3, Medtronic, Inc.) was implanted in a subcutaneous pocket and connected to a pacing electrode inserted via the right jugular vein and positioned endocardially in the right atrium by means of fluoroscopic guidance. A correct positioning of the electrode was verified by atrial stimulation and recording of atrial electrograms and surface ECG. Following pacemaker implantation, the dogs were given postoperative medication (amoxicillin and carprofen) for 3–7 days. At earliest 2–3 weeks after the implantation, the pacemaker was activated and the right atrium paced at a frequency of $\geq$6.9 Hz (depending on ventricular response) for 8 weeks.

Once every week during the RAP period, the pacemaker was temporarily switched off (*Figure 1*). If the dog was in AF and the episode lasted $>$5 min, either AZD2927 (0.67 μmol/kg/min), A7071 (0.13 μmol/kg/min) or saline (1 : 1 : 1 randomization) was intravenously administered for 30 min maximum. At occasions when the pacemaker was switched off and AF was not observed, burst pacing (50 Hz for 5 s) was applied in an attempt to induce AF. If AF lasting $>$5 min could be induced, AZD2927, A7071, or saline was infused as described above. If sinus rhythm was restored by any of the interventions, the infusion was stopped and a blood sample for subsequent analysis of plasma protein binding and drug concentration was drawn. Following successful conversions by A7071, an attempt to re-induce AF by burst pacing (as described above) was immediately undertaken and followed by blood sampling

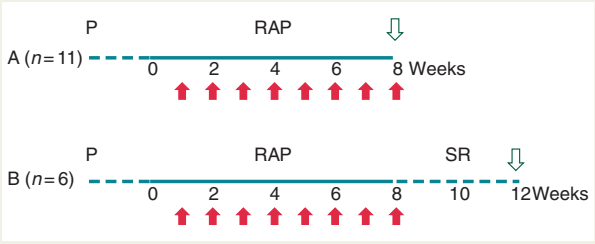

Figure 1 Timing of assessment of antiarrhythmic efficacy and electrophysiological characteristics of AZD2927 and A7071 in dogs. Seventeen dogs were implanted with a right atrial pacemaker (P). Three weeks later the pacemaker was activated to initiate RAP for 8 weeks. Once every week during the RAP period, the pacemaker was temporarily switched off to assess antiarrhythmic drug efficacy (filled arrows). In 11 of the dogs (Group A), an invasive electrophysiological investigation of AZD2927 or A7071 was undertaken after the RAP period (open arrow). A second group of six dogs (Group B) was left in sinus rhythm (SR) for 4 weeks after the 8-week RAP period and subsequently subjected to the invasive investigation. For further details, see the Methods section.

(re-inducibility attempts were not carried out with AZD2927). Subsequently, the pacemaker was turned on and the dog was brought from the laboratory to its normal pen housing.

For estimation of the dominating AF frequency and cycle length, digitized (1 kHz) ECG signals from Lead II were de-trended and de-noised using Coiflet Wavelet algorithms.[11] For each ECG complex the interval from the start of the Q wave to the end of the T wave (QRST) was then cancelled using an average beat subtraction algorithm based on QRST complex detection by template matching. The derived signal was subjected to a discrete Fourier transformation (30 s intervals), displayed as a power spectrum and the dominating frequency was subsequently estimated.

Electrophysiological characterization of AZD2927 and A7071 in the anaesthetized dog

Following the 8-week RAP period, 11 dogs were subjected to an invasive electrophysiological and haemodynamic investigation (group A, *Figure 1*). The primary objective was to assess the potency of AZD2927 ($n = 5$) and A7071 ($n = 6$) increasing the left and right atrial effective refractory period (LAERP and RAERP) in the electrically remodelled atria. The remaining six dogs, which were studied after the clinical data in the atrial flutter patients (see below) became available, were left in sinus rhythm for 4 weeks before a similar investigation was carried out to examine whether the reversal of atrial electrical remodelling would alter the potency of AZD2927 ($n = 3$) and A7071 ($n = 3$) in increasing the LAERP and right atrial effective refractory period (RAERP) (Group B, *Figure 1*). Furthermore, in three of the dogs an investigation to assess the effects of AZD2927 (0.13 μmol/kg/min for 30 min) on LAERP was undertaken the day after the RAP was terminated and repeated 4 weeks later. The intention of these two investigations was to compare the effect of AZD2927 on LAERP in the remodelled and non-remodelled left atrium in the same dog, respectively. At these occasions, the dogs were anaesthetized and instrumented for LAERP measurements as described below.

The dogs were anaesthetized with propofol (8 mg/kg), buprenorphine (0.015 mg/kg), and sodium pentobarbital (4–5 mg/kg/h) and were artificially ventilated. Blood gases and pH in arterial blood were measured and, if necessary, adjusted. The rectal temperature was kept between 37.5°C and 39.2°C by covering the animals and by external heating. Percutaneous catheters for administration of anaesthetics, sodium bicarbonate, Ringer solution, and drugs, respectively, were inserted into the brachial veins. A polyethylene catheter was inserted into the left femoral artery and advanced to the level of the aortic arch for blood pressure recording by means of a pressure transducer and for blood sampling. For recording of right atrial and ventricular electrograms and for atrial and ventricular pacing, two 6F quadripolar electrophysiological recording catheters (Electrophysiology catheter- Deflectable tip, Biosense Webster, Inc, Johnson & Johnson, Diamond Bar) were advanced into the right femoral vein and positioned high up in the right atrium and in the apex of the right ventricle. To reach the left atria for recording and pacing, a 7F quadripolar electrophysiological catheter (Bard Electrophysiology Division, C.R. Bard, Inc., Lowell) was inserted into the left jugular vein, via the right atria, and positioned in the coronary sinus. All electrodes were advanced via introducers (6F and 7F, Radiofocus® Introducer II, TERUMO Europe n.v.) by means of the Seldinger Technique and correctly positioned through fluoroscopic guidance. The RAERP and LAERP and the right ventricular effective refractory period (RVERP) were determined at a stimulation current strength of ~20% above the threshold for pacing the atria and the ventricle, respectively. A custom-made PC-based (AstraZeneca R&D) programmable stimulator and a constant current pulse generator (WPI Stimulus Isolator, World Precision Instruments) were used for stimulation at a basic cycle length (S1-S1) of 350 ms. A premature extrastimulus (S2) was introduced after every 10th paced basic beat with increments of 2 ms until capture. The ERP was defined as the longest S1–S2 interval at which S2 failed to capture.

Needle electrodes were placed subcutaneously to record the surface lead (I, II, III) ECG during atrial pacing. Via the right carotid artery, a 7F Millar Micro-TIP® transducer (SPR-370 Millar instruments, Inc.) was positioned in the left ventricle for measurement of the maximal rate of left ventricular pressure development ($LV_{max}dP/dt$).

The body temperature, the haemodynamic variables, the ECG, and the electrograms were recorded on a personal computer at predetermined intervals. The signals were amplified by custom-made amplifiers and sampled to the computer at a frequency of 200–1000 Hz (different for different variables), and each sampling period lasted for 10 s. Data were processed with PharmLab v.6.0, a custom-made computer program developed for acquisition and analysis of physiological signals measured in animals.

Approximately 1 h after the completion of the animal preparation, pre-drug (control) measurements were carried out. After two control measurements, the dogs were administered AZD2927 (0.044 and 0.21 μmol/kg/min) or A7071 (0.022 and 0.1 μmol/kg/min) as two consecutive 45 min infusions followed by a washout (drug-free) period for ~90 min. Haemodynamic and electrophysiological measurements were repeatedly carried out and arterial blood samples for determination of plasma levels of AZD2927 or A7071 and *ex vivo* plasma protein binding were drawn at predefined time points (for analytical details, see the Supplementary file).

A separate group of seven naïve dogs (not subjected to RAP) were instrumented and prepared basically as described above (LAERP was not measured) and intravenously administered AZD2927 (0.12 and 0.49 μmol/kg/min, $n = 3$) or A7071 (0.02 and 0.05 μmol/kg/min) as two consecutive 45 min infusions followed by a washout (drug-free) period for ~90 min. Haemodynamic and electrophysiological measurements and blood sampling were carried out as described above.

After the invasive investigation was completed, the dog (still under full anaesthesia) was sacrificed with an intravenous overdose of pentobarbital (100 mg/kg). Following the injection heart rate, blood pressure and ECG were monitored.

Pharmacokinetic/pharmacodynamic modelling of electrophysiological variables

Pharmacokinetic and pharmacodynamic analysis and modelling were performed as described in the Supplementary material online, data supplement.

Statistics

Results are presented as means ± SEM, and *n* indicates the number of observations. Student's *t*-test, repeated-measures ANOVA, Dunnet's multiple comparison test, and Fisher's exact probability test were used for statistical evaluation. A *P* value of <0.05 was considered as statistically significant.

Invasive electrophysiological assessment of AZD2927 in atrial flutter patients

The clinical study was carried out in accordance with the ethical principles originating in the Declaration of Helsinki and consistent with International Conference on Harmonization, Good Clinical Practice, applicable regulatory requirements, and the AstraZeneca policy on Bioethics. The final clinical study protocol was approved by national ethics committees and regulatory authorities and registered at www.clinicaltrials.gov (identifier NCT01396226). All patients had to fulfil prespecified inclusion and exclusion criteria including provision of written informed consent before enrollment and/or randomization.

The study was a multi-centre, double-blind, randomized, placebo-controlled study to assess the effects of a single dose of intravenously administered AZD2927 on atrial and ventricular refractoriness in patients undergoing an invasive electrophysiological procedure. The main purpose of the study was to validate I_{KACh} as an atrial-selective target for rhythm control of AF built on the findings in animals. Patients enrolled into the study were males or postmenopausal females, aged 20–80 years and with a clinical indication for catheter ablation of atrial flutter. Single episodes of persistent atrial flutter or AF requiring cardioversion did not exclude the patient from participating, but the patients had to be in sinus rhythm at randomization. Adequate anticoagulation or antithrombotic treatment according to national guidelines was mandatory.

The electrophysiological investigation was undertaken before, but in conjunction with catheter ablation of atrial flutter. The rationale for undertaking the electrophysiological investigation of AZD2927 first was based on the fact that the ablation *per se* may affect the autonomic tone and, thus, the pharmacodynamic effects of AZD2927 as the I_{KACh} is partly under vagal influence. Prolongation of the primary variable assessed in this study, LAERP, is considered a surrogate marker of atrial antiarrhythmic efficacy in rhythm control and changes of LAERP were regarded as adequate for evaluation of AZD2927. AZD2927-induced changes of RVERP and the paced QT interval were assessed as secondary variables in order to study whether AZD2927 had any effects on ventricular refractoriness or repolarization.

AZD2927 or placebo was infused according to a bolus (15 min infusion, 120 mL/h) and maintenance infusion (45 min maximum, 45 mL/h) regimen. For AZD2927, the dosing rate was 60 mg/kg (bolus infusion) and 23 mg/h (maintenance infusion) and aimed at obtaining a pseudo steady-state plasma concentration of 1.5 μmol/L at the end of the infusion, a concentration predicted to increase LAERP by 20–30 ms. The infusion was stopped when all electrophysiological measurements had been carried out or after 60 min infusion maximum.

The patient was brought to the electrophysiology laboratory in a fasting, non-sedated state. Catheters were introduced percutaneously into a femoral vein and into the left brachial vein, left subclavian vein, or right internal jugular vein. The LAERP and RVERP were measured from the coronary sinus and the right ventricular apex, respectively. Electrocardiograms were recorded in sinus rhythm and at a drive cycle length of 600 ms, whereas LAERP and VERP were measured using drive trains of eight beats at 500 ms and single premature stimuli with progressively shortened coupling intervals in 10 ms decrements, until failure to capture. All measurements were undertaken in duplicate. The electrophysiological assessments along with pharmacokinetic sampling were carried out before the start of administration of the investigational products and during the infusion starting 30 min after its initiation. The assessment of LAERP was repeated at the end of the infusion period.

Safety measures included adverse events, ECG (including heart rate), blood pressure, physical examination, weight, and laboratory variables. Short episodes (<5 min) of AF, atrial flutter, and other supraventricular tachyarrhythmias were not regarded as adverse events and not a reason for discontinuation of study drug administration. Following the ablation, continuous ECG monitoring was carried out by telemetry until discharge the next day.

Statistics

The primary analysis of the primary variable, LAERP, was based on a paired *t*-test of the mean difference from baseline for the dose group of patients randomized to AZD2927. The estimated true mean change is reported, together with a 95% confidence interval and a *P* value, calculated using Student's *t* distribution, for testing the null hypothesis that the true change was zero.

Results

Preclinical studies

Ion channel-blocking effects *in vitro*

AZD2927 and A7071 concentration-dependently blocked I_{KACh} in human atrial myocytes with IC_{50} values for block of 0.35 ± 0.16 and 0.59 ± 0.21 μmol/L (*Table 1*). The potencies were similar to the potencies estimated in the CHO cells expressing the human isoform of the channel protein. Furthermore, both compounds showed selectivity against other repolarizing and depolarizing currents and did not inhibit the muscarinic M2 receptor.

Table 1 Ion channel-blocking profile of AZD2727 and A7071 in human atrial myocytes or CHO cells and muscarinic M2 receptor inhibition in CHO cells

Ion current/channel/ receptor	AZD2927 (IC_{50}, μmol/L)	A7071 (IC_{50}, μmol/L)
I_{KACh} (human atrial myocyte)	0.35 ± 0.16	0.59 ± 0.21
Kir3.1/Kir3.4/M2 (I_{KACh}, CHO)	1.3 ± 0.1	1.6 ± 0.3
hERG (I_{Kr}, CHO)	>100	>33
hKv1.5 (I_{Kur}, CHO)	>33	ND
hKv4.3 (I_{to}, CHO)	>100	>100
hKvLQT1/hminK (I_{Ks}, CHO)	>100	>100
hNav1.5 (I_{Na}, CHO)	100	>100
hCav1.2 ($I_{Ca,\,L}$, CHO)	>100	>100
hM2 receptor (CHO)	>33	>33

Data given as best-fit values ± SEM. Curves were fitted to replicate data using non-linear regression by means of a four-parameter logistic (curve top, slope, and IC_{50} variable.
ND, not determined.

Antiarrhythmic efficacy of AZD2927 and A7071 during 8 weeks of rapid atrial pacing

Seventeen dogs were subjected to RAP for 8 weeks. On a weekly basis, the pacemaker was turned off and the presence, inducibility, and re-inducibility of AF were examined. In total, 41 episodes of AF lasting >5 min were observed. At these instances, either AZD2927, A7071 or saline was infused for 30 min maximum in an attempt to restore sinus rhythm. AZD2927 successfully restored sinus rhythm at 10/10 attempts and A7071 at 14/14 attempts, whereas saline converted 4/17 episodes only ($P < 0.001$ vs. AZD2927 and A7071, *Figure 2*). In the AZD2927-treated dogs, the time to conversion was 266 s (range 144–370 s) and occurred at an unbound plasma concentration of 1.6 ± 0.20 μmol/L. In the A7071-infused dogs, the time to conversion was 390 s (range 25–995 s) and conversion occurred at an unbound plasma concentration of 0.7 ± 0.08 μmol/L. In the saline-administered dogs, the time to the four successful conversions was 222 s (range 8–733 s). The infusion of AZD2927 and A7071 was associated with a progressive reduction in AF frequency. Hence, immediately before AF conversion, the AF frequency had decreased from 12.9 ± 0.9 to 10 ± 1.7 Hz ($P < 0.001$) and from 13.3 ± 1.2 to 11.3 ± 1.1 Hz ($P < 0.001$) in the AZD2927- and A7071-infused dogs, respectively. During saline infusion, the AF frequency did not significantly change (from 13.7 ± 0.1 to 13.4 ± 0.3 Hz). Following the conversion of AF by A7071, an attempt to re-induce AF was promptly carried out by burst pacing. At only 3 out of the 14 attempts, AF could be re-induced ($P < 0.001$ vs. the inducibility prior to A7071 infusion). Immediately after the inducibility attempt, the unbound plasma concentration of A7071 was 0.5 ± 0.07 μmol/L.

Electrophysiological characterization of AZD2927 and A7071 in the anaesthetized dog

All 17 dogs included in the study underwent a terminal invasive electrophysiological and haemodynamic investigation to assess the effects of AZD2927 and A7071. Eleven of the dogs (Group A) were examined following 8 weeks of RAP, of these five and six dogs received AZD2927 or A7071, respectively. In a second group (Group B), six dogs were investigated after 8 weeks of RAP followed by 4 weeks in sinus rhythm in an attempt to assess drug effects following reversal of the atrial electrical remodelling induced by the RAP. In the latter group, three dogs each were administered AZD2927 and A7071. Before administration of the drugs, baseline LAERP was significantly longer (139 ± 7 ms vs. 107 ± 7 ms, $P < 0.01$) in the dogs left in sinus rhythm after the pacing period compared with the dogs studied immediately after the RAP period (*Table 2*). Likewise, RAERP tended to be longer in the former group than in the latter (149 ± 9 vs. 135 ± 7 ms, NS). Neither the RVERP (168 ± 5 ms vs. 167 ± 2 ms, respectively) nor the QT interval (176 ± 4 ms vs. 167 ± 4 ms) differed in the two groups of dogs. Mean aortic blood pressure, $LV_{max}dP/dt$, and the QRS interval were similar in both the groups of dogs, whereas heart rate was significantly higher in dogs studied immediately after RAP (133 ± 6 vs. 97 ± 6 b.p.m., $P < 0.01$, *Table 2*).

For both AZD2927 and A7071, the infusion regimens adopted gave rise to similar exposure levels in dogs studied after the RAP period and in dogs studied 4 weeks later having been in sinus rhythm (*Figures 3A* and *4A*, respectively). The infusion of AZD2927 and A7071 was associated with a concentration-dependent increase in RAERP and LAERP (*Figures 3B* and *C* and *4B* and *C*). The changes were similar in the dogs studied after the pacing period and in the dogs studied following pacing and sinus rhythm. In contrast to the increase in atrial refractoriness, RVERP and the QT interval were only minimally altered indicative of an atrial-selective electrophysiological profile of both compounds. For A7071, the PK/PD modelling predicted an unbound plasma concentration to increase LAERP and RAERP by 30 ms of 0.38 and 1.05 μmol/L in dogs studied after the RAP period and of 0.57 and 0.81 μmol/L in the dogs assessed 4 weeks after the pacing was stopped. In the AZD2927-treated dogs, the corresponding predicted unbound concentrations for the RAERP change were 0.25 and 0.61 μmol/L, respectively. For the LAERP

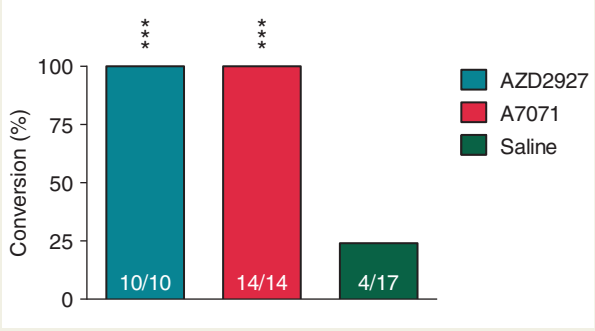

Figure 2 Conversion of AF to sinus rhythm by AZD2927, A7071, and saline in dogs ($n = 17$) subjected to rapid atrial pacing (RAP) for 8 weeks. For AF episodes lasting >5 min, AZD2927 (0.67 μmol/kg/min), A7071 (0.13 μmol/kg/min), or saline (1 : 1 : 1 randomization) were intravenously administered for 30 min maximum in an attempt to restore sinus rhythm. Numbers within bars indicate episodes of AF successfully converted to sinus rhythm. ***$P < 0.001$ vs. saline (Fisher's exact probability test).

Table 2 Baseline electrophysiological and haemodynamic characteristics in dogs subjected to 8 weeks of rapid pacing or 8 weeks of rapid atrial pacing followed by sinus rhythm for 4 weeks

Variable	RAP ($n = 11$)	RAP + SR ($n = 6$)
LAERP (ms)	107 ± 7	139 ± 7[a]
RAERP (ms)	135 ± 7	149 ± 9
RVERP (ms)	167 ± 2	168 ± 5
QRS (ms)	43 ± 1	42 ± 1
QT (ms)	167 ± 4	176 ± 4
HR (b.p.m.)	133 ± 6	97 ± 6[b]
AOP (mmHg)	125 ± 4	123 ± 4
$LV_{max}dP/dt$ (mmHg/s)	2675 ± 112	2761 ± 163

RAP, rapid atrial pacing for 8 weeks; RAP + SR, rapid atrial pacing for 8 weeks followed by sinus rhythm for 4 weeks; LAERP, left atrial effective refractory period; RAERP, right atrial effective refractory period; RVERP, right ventricular effective refractory period; HR, heart rate; AOP, mean aortic blood pressure; $LV_{max}dP/dt$; maximal left ventricular pressure development. All variables (except HR) were recorded or measured at a basic cycle length of 350 ms.
[a]$P < 0.05$ vs. RAP.
[b]$P < 0.01$ vs. RAP.

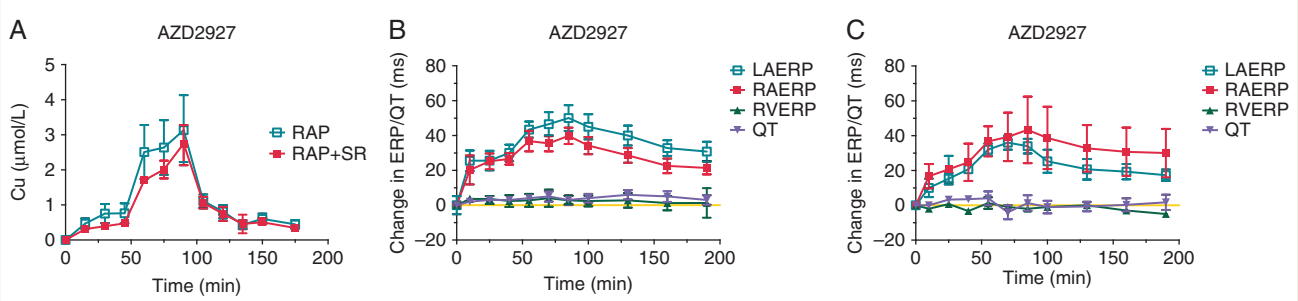

Figure 3 (*A*) Unbound plasma concentrations of AZD2927 (Cu) in two groups of dogs administered AZD2927 as two consecutive 45 min infusions (0.044 and 0.21 μmol/kg/min) followed by a washout (drug-free) period for ~90 min. The dogs were studied immediately after 8 weeks of RAP (open squares, $n = 5$) or after 8 weeks of rapid atrial pacing followed by 4 weeks of sinus rhythm (filled squares, $n = 3$). (*B*) Absolute changes in the left and right atrial effective refractory period (left atrial effective refractory period, LAERP, and RAERP) and in right ventricular effective refractory period (RVERP) and the QT interval (QT) assessed immediately after 8 weeks of rapid atrial pacing. (*C*) Absolute changes in LAERP, RAERP, RVERP, and QT interval assessed immediately after 8 weeks of rapid atrial pacing followed by 4 weeks of sinus rhythm.

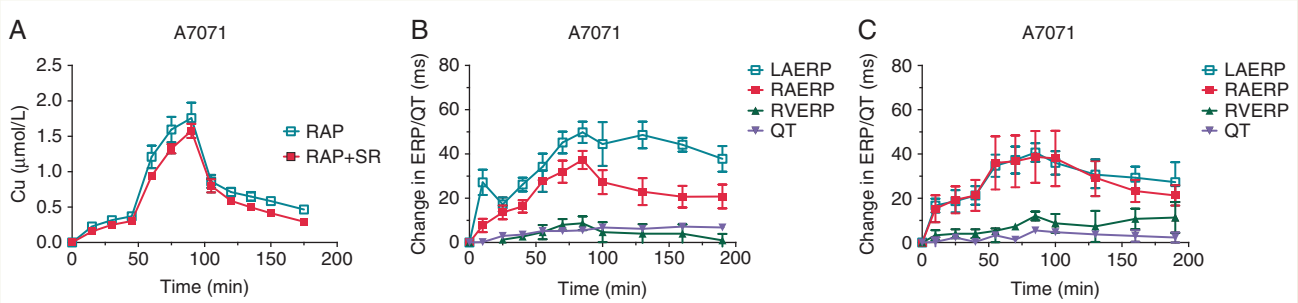

Figure 4 (*A*) Unbound plasma concentrations of A7071 (Cu) in two groups of dogs administered A7071 as two consecutive 45 min infusions (0.022 and 0.1 μmol/kg/min) followed by a washout (drug-free) period for ~90 min two consecutive 45 min intravenous drug infusions followed by a washout for 90 min. The dogs were studied immediately after 8 weeks of rapid atrial pacing (open squares, $n = 5$) or after 8 weeks of rapid atrial pacing followed by 4 weeks of sinus rhythm (filled squares, $n = 3$). (*B*) Absolute changes in the left and right atrial effective refractory period (LAERP and RAERP) and in right ventricular effective refractory period (RVERP) and the QT interval (QT) assessed immediately after 8 weeks of rapid atrial pacing. (*C*) Absolute changes in LAERP, RAERP, RVERP and QT interval assessed immediately after 8 weeks of rapid atrial pacing followed by 4 weeks of sinus rhythm.

change, the predicted unbound concentration was 0.42 μmol/L in the three dogs examined after the pacing period, whereas the unbound concentration could not be accurately predicted in the dogs subjected to pacing followed by sinus rhythm due to poor precision and clockwise hysteresis. No consistent drug-related changes were observed on the QRS interval, heart rate, mean aortic blood pressure, or $LV_{max}dP/dt$ (data not shown).

In three of the dogs included in the second study group (Group B), the effects of AZD2927 in increasing LAERP was examined immediately following 8 weeks of RAP. The investigation was then repeated after 4 weeks, a period during which the dogs were in sinus rhythm. Immediately after the RAP period, the baseline (pre-drug) LAERP was 115 ± 20 ms and following 4 weeks in sinus rhythm 157 ± 23 ms, a difference that clearly indicates reversal of left atrial electrical remodelling. The infusion of AZD2927 resulted in quantitatively similar exposures and absolute increases in LAERP at the two study occasions (*Figure 5*). However, the relative increase in LAERP tended to be more pronounced when assessed immediately after RAP vs. 4 weeks later. PK/PD modelling of the data predicted a slightly lower unbound plasma concentration of AZD2927 to increase the LAERP by 30 ms when assessed immediately after the RAP period when compared with the assessment 4 weeks later (0.5 ± 0.14 vs. 1.0 ± 0.71 μmol/L).

In seven naïve dogs, the infusion of AZD2927 ($n = 3$) or A7071 resulted in a concentration-dependent increase in RAERP. The PK/PD modelling predicted an unbound plasma concentration of AZD2927 and A7071 to increase the RAERP by 30 ms of 0.30 and 0.20 μmol/L, respectively. These predicted values are similar to those in the dogs studied after the RAP period and in the dogs assessed 4 weeks after the pacing was stopped.

Invasive electrophysiological assessment of AZD2927 in atrial flutter patients

A total of 18 patients (*Table 3*) were randomized of which 12 patients received AZD2927. All patients who received treatment completed

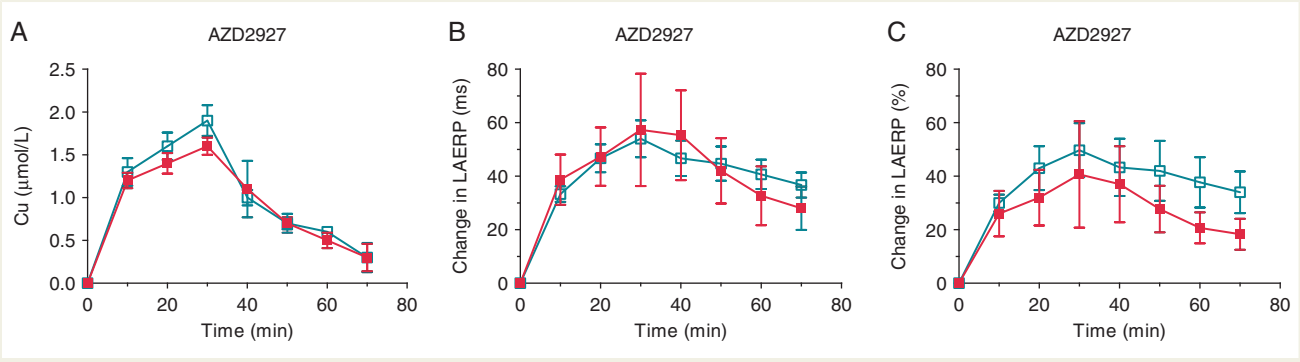

Figure 5 Unbound plasma concentration (Cu, *A*) and absolute (*B*) and relative (*C*) changes in the left atrial effective refractory period (LAERP) in three dogs administered a 30 min continuous intravenous infusion of AZD2927 (0.13 µmol/kg/min) followed by washout for 30 min. The dogs were studied immediately after 8 weeks of rapid atrial pacing (open symbols) and then a second time after a period of 4 weeks during which the dogs were in sinus rhythm (filled symbols).

Table 3 Patient demographics and baseline characteristics

Demographic characteristics	AZD2927 (n = 12	Placebo (n = 6)	Total (n = 18)
Age (year) (mean)	57.9	63.8	59.9
Male/female	11/1	5/1	16/2
Weight (kg) [mean (range)]	88.8 (70–122)	85.5 (61–106)	87.7 (61–122)
BMI (kg/m^2) [mean (range)]	26.4 (21–33)	26.7(21–33)	26.5 (21–33)
Left atrial size (cm^2) [mean (range)]	24.8 (22–28)	25.5 (17–30)	25.1 (17–30)
Right atrial size (cm^2) [mean (range)]	22.2 (19–25)	22.7 (18–28)	22.4 (18–28)
LVEF (%) [mean (range)]	59.2 (55–65)	58.3 (50–65)	58.9 (50–65)
Atrial flutter (duration since the first episode diagnosed (years) [mean (range)]	2.0 (0–8)	3.1 (0–5)	2.4 (0–8)
Atrial flutter (duration since most recent episode (days) [mean (range)]	93.2 (31–218)	201.3 (4–553)	129.2 (4–553)
AF [duration since first episode diagnosed (years)] [mean (range)]	11.0 (3–19)	11.3 (0–26)	11.2 (0–26)
AF [duration since most recent episode (days)] [mean (range)]	297.3 (152–568)	923.3(23–2722)	610.3 (23–2722)
Asthma or other obstructive pulmonary disease (past/current)	0/1	0/0	0/1
AF (past/current)	1/2	2/1	3/3
Atrial flutter (past/current)	4/8	4/2	8/10
Hypertension (past/current)	0/4	0/4	0/8
Congestive heart failure (past/current)	0/0	0/0	0/0
Beta-blocker (n)[a]	7	3	10
ACE inhibitor + ARB (n)[a]	4	3	7
Calcium blocker (n)[a]	1	1	2

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor 1 blocker; BMI, body mass index; LVEF, left ventricular ejection fraction.
[a]Concomitant medication after study entry.

the study and were included in the efficacy and safety analyses. One patient was excluded from the per protocol analysis set as the AZD2927 administration did not comply with the method defined in the study protocol. In the two groups of patients, median left atrial size, ejection fraction, and time elapsed since the last atrial flutter episode were 25 cm^2, 60% and 72 days (AZD2927) and 28 cm^2, 60% and 825 days (placebo), respectively. None of the AZD2927 patients had a documented atrial flutter episode within the last 30 days prior to randomization.

The plasma concentrations of AZD2927 rapidly increased in a linear fashion from the start of the infusion until 15 min and then remained relatively constant during the maintenance infusion (with only a small increase at the second and last assessment of LAERP). At the time of the last assessment of LAERP the total plasma concentration was 1.2 ± 0.41 µmol/L. The primary objective of this study was to evaluate the effects of AZD2927, compared with baseline, on LAERP. The changes from baseline are illustrated in *Figure 6*. The mean difference from baseline at the last

LAERP assessment in the AZD2927 group was 2.7 ms (95% CI −4.1 to 9.5, $P = 0.3911$, *Table 4*). In the placebo group, the corresponding change was 8.2 ms (95% CI −24.8 to 41.2, $P = 0.5525$). Neither infusion of AZD2927 nor placebo was associated with any change in RVERP or paced QT interval (*Table 4*).

AZD2927 was considered safe and well tolerated. No serious adverse events were reported and the few adverse events observed were equally distributed among AZD2927-treated and placebo-treated patients. One patient in the placebo group discontinued study drug infusion prematurely due to an episode of AF. No clinically relevant changes were seen for the laboratory variables, vital signs, or ECG variables.

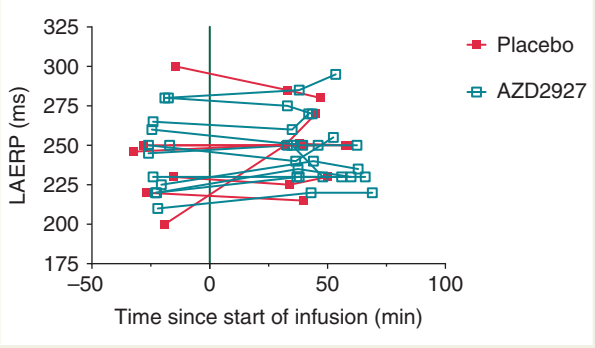

Figure 6 Change in left atrial effective refractory period (LAERP) from baseline in patients administered a continuous infusion of AZD2927 ($n = 12$, open squares) or placebo ($n = 6$, filled squares). AZD2927 or placebo was infused according to a bolus (15 min infusion, 120 mL/h) and maintenance infusion (45 min maximum, 45 mL/h) regimen. For AZD2927 the dosing rate was 60 mg/kg (bolus infusion) and 23 mg/h (maintenance infusion) aiming at obtaining a pseudo steady-state plasma concentration of 1.5 μmol/L at the end of the infusion. Left atrial effective refractory period was assessed prior to the start of infusion and twice during the maintenance infusion.

Discussion

The major objective of the present series of experiments was to examine the potential of I_{KACh} as a viable and atrial-selective target for treatment of atrial tachyarrhythmias. Based on *in vitro* assessments of their ion channel-blocking potency, AZD2927 and A7071 were considered selective I_{KACh}-blocking antiarrhythmic agents and brought forward further studies in dogs and humans (AZD2927 only). To the best of our knowledge, this is the first study invasively assessing the electrophysiological characteristics of a selective I_{KACh}-blocking agent in atrial arrhythmia patients.

Dogs subjected to RAP for extended periods of time have frequently been used for assessing antiarrhythmic drug efficacy and the model has many characteristics in common with the AF patient.[12] A typical consequence of the rapid atrial rate whether in animals or patients is a pronounced abbreviation of the atrial refractory period.[11–13] This fundamental alteration in atrial electrophysiology does not only increase AF vulnerability but may also have considerable implications for the electrophysiological actions of antifibrillatory drugs. For example, in goats with remodelled atria it was found that dofetilide and ibutilide, two agents that selectively and potently block the rapid delayed rectifying potassium current (I_{Kr}), partly lost their potential to increase atrial refractoriness and could not prevent AF inducibility.[14] The opposite was true for AVE0118, an agent with preferential blocking effect on atrial-selective repolarizing potassium currents (I_{Kur} and I_{KACh}), which markedly prolonged refractoriness and possessed antiarrhythmic action in the remodelled atrium. Likewise, preserved electrophysiological effects and antiarrhythmic efficacy were recently demonstrated for the I_{Kur}-blocking agents MK-0448 and AZ13395438.[5,11]

Acetylcholine stimulates muscarinic M2 receptors and activates atrial I_{KACh} which causes shortening of the atrial APD which in turn creates a substrate for AF induction and perpetuation.[15] In addition, sustained AF leads to development of an inward constitutively active potassium current (with properties of I_{KACh}) being active in the absence of muscarinic receptor activation.[3,8] Such a constitutively active I_{KACh} has been shown to contribute to an increased basal inward current in dogs subjected to atrial tachypacing as well as in

Table 4 Changes from baseline in the left atrial and right ventricular effective refractory period and the paced QT interval following infusion of AZD2927 or placebo in patients undergoing an invasive electrophysiological investigation

	Before infusion		Last assessment				
Treatment	***N***	**Mean (SD)**	***N***	**Mean (SD)**	**Difference**	**95% CI**	***P* value**
AZD2927 (LAERP)	11	246 (24.5)	11	249 (22.7)	2.7 (10.1)	(−4.1, 9.5)	0.391
Placebo (LAERP)	6	241 (34.1)	6	249 (24.2)	8.2 (31.4)	(−24.8, 41.2)	0.553
AZD2927 (VERP)	11	225 (23.8)	11	224 (17.5)	−0.9 (9.4)	(−7.3, 5.4)	0.756
Placebo (VERP)	6	218 (21.4)	6	220 (21.0)	1.7 (7.5)	(−6.2, 9.6)	0.611
AZD2927 (QT)	11	356 (32.3)	11	355 (31.0)	−1.0 (9.1)	(−7.1, 5.1)	0.735
Placebo (QT)	6	390 (29.5)	6	369 (31.2)	6.3 (8.4)	(−2.5, 15.1)	0.124

LAERP, left atrial effective refractory period; VERP, ventricular effective refractory period. LAERP and VERP were measured at a drive cycle length of 500 and the QT interval at 600 ms, respectively.

right and left atrial tissue from patients with long-lasting AF.[8,16] In electrically remodelled dog atria and in rat atrial cardiomyocytes, selective blockade of the constitutively active I_{KACh} current was associated with prolongation of the APD and suppression of inducible AF and the particular current has actually been suggested a novel therapeutic target in AF.[3,8–10,17] In the present series of experiments in the dog, we applied a PKPD modelling approach to predict the unbound plasma concentration of AZD2927 and A7071 increasing the atrial effective refractory periods by 30 ms, an increase assumed to translate into antiarrhythmic efficacy. Based on the PKPD modelling of the exposure and efficacy data, the potency of both drugs was surprisingly found to be similar in dogs studied immediately after tachypacing and in dogs left in sinus rhythm to reverse the electrical remodelling induced by the tachypacing. This is partly in agreement with *in vitro* observations by Cha *et al.* who found that the selective I_{KACh} blocker tertiapin-Q prolonged to a similar extent the action potential duration in cardiomyocytes isolated from coronary-perfused left atrial preparations of tachypaced and in myocytes from control dogs.[9] However, as the rapid pacing procedure was associated with an abbreviation of the atrial action potential duration, the relative increase was significantly larger than the absolute increase. In contrast, Koo *et al.*[18] did not find any action potential duration prolongation by tertiapin-Q in normal atrial myocytes but significant increases in the electrically remodelled myocyte. In line with our findings demonstrating an atrial-selective action of AZD2927 and A7071, tertiapin-Q demonstrated a selective electrophysiological profile as ventricular action potential duration was unaltered. Based on our results in naïve and paced dogs *in vivo* as well as the ion channel-blocking profile of AZD2927 and A7071 *in vitro*, it is likely that blockade of constitutively active I_{KACh} contributes to the observed increase in atrial refractoriness.

The efficacy of AZD2927 and A7071 in restoring sinus rhythm and inhibiting re-inducibility (studied for A7071 only) of AF was assessed weekly in the conscious dog during the 8-week rapid pacing periods. When compared with the vehicle (saline), both agents were significantly more efficacious in rapidly converting AF and effectively inhibiting re-induction of AF. Conversion was preceded by a significant increase in AF cycle length which is in line with previous findings for other agents blocking atrial repolarizing currents.[11,14] Based on the observation that the unbound plasma concentration of AZD2927 and A7071 at the time of the conversion fell within the ranges of the unbound plasma concentrations increasing the atrial effective refractory periods by 30 ms, one may speculate that in the tachypaced dog model of AF, such an increase in refractoriness may suffice to restore sinus rhythm in the fibrillating atria. Our results are very much in line with observations from studies on NTC-801, a highly selective I_{KACh} blocker, which was found to increase atrial refractoriness by 35–40 ms at doses associated with significant reductions in AF inducibility in dogs atrially tachypaced for 3–5 weeks.[4,19]

The major purpose of the clinical study of AZD2927 in atrial flutter patients scheduled for an ablative procedure was to examine its safety and tolerability and pharmacokinetics and to verify the electrophysiological action seen in dogs. AZD2927 was considered as safe within the concentration ranges studied and the adverse events were equally distributed among placebo- and AZD2927-treated subjects. The patients had experienced atrial flutter in the past while relatively few had a diagnosis of past or current AF. Since patients with paroxysmal atrial flutter also have episodes of AF, or develop AF over time, the study population was considered similar to the intended future target population for an I_{KACh}-blocking drug, i.e., patients with symptomatic AF. The study procedure was scheduled to take place before the ablation as I_{KACh} is partly under vagal influence and as the procedure may affect the autonomic tone and thus the response to AZD2927. Toivonen *et al.*[20] recently demonstrated that AZD1305, an antiarrhythmic agent predominantly blocking the delayed rectifying potassium current and the sodium current, concentration-dependently increased atrial refractoriness in an identical atrial flutter patient population as examined in the present study. These observations thus support the rationale for including atrial flutter patients in studies invasively assessing electrophysiological characteristics of novel antiarrhythmic agents.

In the patients, the infusion of AZD2927 was not associated with any alterations in ventricular repolarization or refractoriness, observations in line with the findings in the dog. Surprisingly, however, AZD2927 did not increase LAERP in patients, a finding in sharp contrast with the prominent change in the dogs. One may argue that the lack of effects was a result of the fact that the patients randomized to the study had been in sinus rhythm for rather long periods (>30 days). Consequently, the electrical remodelling may have been reversed and the activity of constitutively open I_{KACh} channels attenuated resulting in a reduced potency of AZD2927. In the dogs, however, AZD2927 as well as A7071 were almost equally effective in increasing atrial refractoriness in remodelled atria as in atria studied after reversal of electrical remodelling which speaks against this explanation. The AZD2927 dosing rate in the patient study was estimated from pharmacokinetic characteristics in the healthy subjects as well as PKPD relationships in the tachypaced dogs. Based on these relationships, the target pseudo steady-state plasma concentration of 1.5 μmol/L was predicted to result in a mean LAERP increase from baseline of 20 to 30 ms. Although this target concentration was not fully reached in the present study, it is highly unlikely that a too low exposure may underlie the complete lack of effect on the primary variable. Furthermore, plasma protein binding of AZD2927 in the dog and in man is similar (66% and 68%, respectively) excluding prominent differences in unbound drug concentrations. Other more plausible explanations for the discrepancy in effect between the dog and the man may include potential species differences in regional channel protein expression and current levels including a much higher magnitude of constitutively active I_{KACh} in dogs vs. man. This hypothesis is supported by our observation that both study drugs concentration-dependently increased atrial refractoriness in naïve control dogs.[8,9,21] Interestingly, Pavri *et al.*[5] recently demonstrated that MK-0448, a potent blocker of I_{Kur} with minimal off-target activity *in vitro* and selective action on atrial vs. ventricular electrophysiology and antiarrhythmic efficacy in anaesthetized dogs *in vivo*, did not increase atrial refractoriness in young healthy subjects. Their observations and our findings illustrate the difficulties in translating results from animals to man and that information generated in animal models should be interpreted cautiously.

Study limitations

In the present study, a frequently used dog model of atrial remodelling and AF was adopted to assess the electrophysiological and

antiarrhythmic characteristics of two novel I_{KACh} blockers, AZD2927 and A7071. For other antiarrhythmic agents, the model has demonstrated good translatability towards AF patients which was not the case in the present study enrolling patients with a history of atrial flutter. In the dog, both compounds were equally effective in increasing atrial refractoriness in normal as well as in electrically remodelled atria which may be interpreted as block of constitutively active I_{KACh} in both cases. Ideally and for comparison, the *ex vivo* effects of the compounds should have been assessed in atrial cardiomyocytes isolated from patients in sinus rhythm and in patients with sustained AF. In the atrial flutter patients, however, AZD2927 did not influence atrial refractoriness which may raise concern regarding translatability and the potential of I_{KACh} as an antiarrhythmic target. A drawback is that only one dosing regimen, for which the upper exposure limit was set based on toxicology findings, was explored and it is thus unclear whether increasing the exposure would have resulted in increased refractoriness. One may also argue that atrial flutter patients do not belong to the primary target population and that AF patients would have been more appropriate to study. However, based on past experience we considered atrial flutter patients suitable for this kind of invasive electrophysiological characterization.[20] In retrospect one may argue that the time span between the most recent arrhythmia episode and the actual procedure was very long and the atrial electrophysiology documented not truly representative of that in a patient with more recent or ongoing arrhythmia. For the specific target of interest in the present study, clinical proof-of-principle is still pending and the ultimate study would include patients with ongoing arrhythmia (conversion to sinus rhythm) or a population at high risk of recurring AF.

Conclusion

In the present study, we have demonstrated that selective blockade of I_{KACh} by AZD2927 and A7071 increases atrial refractoriness in electrically remodelled and non-remodelled atria, causes minimal effect on ventricular refractoriness and repolarization and is associated with antiarrhythmic efficacy in the dog. However, these atrial electrophysiological actions could not be verified in the patient population included in the present study and it is thus still an open question whether I_{KACh} is a viable antiarrhythmic target.

Supplementary material

Supplementary material is available at *Europace* online.

Conflict of interest: A.B., L.F., G.L., A.C.N., M.S., and L.C. are current employees of AstraZeneca R&D, CVMD Innovative Medicine, Mölndal, Sweden. The other authors have no conflicts to report.

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