Evaluation of KCB-328, a new \(I_{Kr}\) blocking antiarrhythmic agent in pacing induced canine atrial fibrillation

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Abstract

Hypothesis
KCB-328 is a new potassium channel blocker, which prolongs action potential duration with exhibition of minimal reverse use dependence. We tested the efficacy and proarhythmic potential of KCB-328, dofetilide and propafenone in the pacing induced canine model of atrial fibrillation (AF).

Methods
Montreal dogs in complete heart block were paced for 1–6 weeks to produce AF, and given KCB-328 or dofetilide. A subset then received propafenone 14 ± 3 days after testing the first drug.

Results
KCB-328 prolonged right and left atrial (RA and LA) activation times and AF cycle length (CL), terminating AF in 3 of 6 dogs. RA effective refractory period (ERP) and ventricular ERP and QT interval were prolonged. Dofetilide terminated AF in 1/6 dogs, and given KCB-328 or dofetilide. A subset then received propafenone 14 ± 3 days after testing the first drug.

Conclusion
The spectrum of effect of the three drugs differed significantly: propafenone showed the greatest success in AF termination, and both propafenone and KCB-328 appeared less proarhythmic than dofetilide in this model.

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KEYWORDS
chronic heart block; antiarrhythmic drugs; potassium channel blockade; proarhythmia; effective refractory period; atrial fibrillation

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Introduction

Atrial fibrillation (AF) occurs in 5% of the population over age 65 [1]. Pharmacological agents remain the mainstay of therapy for restoration and maintenance of sinus rhythm and for ventricular rate control. Most of the antiarrhythmic agents used for cardioversion or prevention of recurrences of AF increase atrial refractoriness [2]. Of these, agents like dofetilide, sotalol, and ibutilide are potent IKr blockers that significantly prolong action potential duration (APD) [3–5]. These agents also exhibit reverse use dependence, such that their efficacy in prolonging repolarization is more marked at slower rates, increasing the risk for lethal arrhythmias such as torsades de pointes. Their reduced slower rates, increasing the risk for lethal arrhythmias like atrial fibrillation. Na channel blockers like propafenone and flecainide [2] are also important in the treatment of atrial fibrillation. These use-dependent antiarrhythmics have a prominent effect of slowing conduction, although they also prolong refractoriness [2].

The need for additional antiarrhythmic therapies for atrial fibrillation prompted us to investigate the effects of KCB-328 (1-(2-amino-4-mathanesulfonamidophenoxy)-2-(N-(3,4-dimethoxyphenethyl)-N-methylamino)ethane hydrochloride), a novel IKr blocker that prolongs APD without exhibiting significant reverse use dependence [6]. We report here its effects on atrial fibrillation and its proarhythmic potential in a conscious canine model. As comparison drugs we used dofetilide, an IKr blocker which is increasingly used in the treatment of atrial fibrillation [7,8] and the sodium channel blocker, propafenone [2].

Methods

The study conformed to the rules of the Columbia University Institutional Animal Care and Use Committee. Twelve female mongrel dogs weighing 25 ± 3 kg were anesthetized with thiopental sodium (17 mg/kg i.v.) and ventilated with isoflurane (1.5–2%) and oxygen (2 l/min). Morphine sulphate (0.15 mg/kg) was injected epidurally for postoperative analgesia. Using sterile techniques, a right intercostal thoracotomy was performed, and the heart suspended in a pericardial cradle. Bipolar pacing leads (Medtronic model 5058) were attached epicardially to the left atrial appendage (LAA) and the right ventricular free wall (RV). Leads were tunnelled subcutaneously, the LAA lead connected to a Medtronic Itrel pulse generator and the RV lead to a single chamber pacemaker (Medtronic Model Thera SR 8962), both of which were implanted in subcutaneous pockets on the posterior chest wall. Additionally, bipolar electrodes for pacing and recording were attached to the right atrial appendage (RAA), LAA and RV, left ventricular apex (LVA) and right ventricular outflow tract (RVOT), tunnelled subcutaneously to the right posterior thorax and exteriorized. Complete heart block was induced by injecting 0.1–0.3 ml of 37% formalin into the atrioventricular (AV) node region [9], after which an escape rhythm of 30–50 bpm ensued. The ventricular pacemaker was programmed at a rate of 60 bpm.

After surgery the dogs were monitored for 2–3 days in the recovery room. They were treated prophylactically with cefazolin, 25 mg/kg i.v. once before surgery and twice daily for 2 days after surgery. The animals were allowed to recover for 3 weeks during which time they were laboratory trained, while lying on their left sides to allow experimentation in the conscious state, and monitored for electrical stability by measuring atrial and ventricular effective refractory periods.

Experimental protocol

When recovery was complete a baseline electrophysiological study was performed to measure effective refractory periods (ERPs) and record ECGs. Thereafter, rapid atrial pacing at 900 bpm was initiated. Animals were studied in the conscious state while lying quietly on their left sides, at weekly intervals. During each study period atrial and ventricular ERPs were measured and ECG recorded to enable measurement of QT intervals. Atrial pacing was continued for 1–6 weeks until the animal consistently had episodes of AF ≥30 min.

When atrial fibrillation continued for 30 min or more after stopping rapid atrial pacing, drug administration was started at the lowest dose. Six dogs were randomized to KCB-328 administration and six to dofetilide. Following recovery from these protocols and restabilization of atrial fibrillation (14 ± 3 days) propafenone was administered to nine of the dogs, selected as follows: three dogs treated with KCB328 and three treated with dofetilide, in which AF did not terminate were treated with propafenone as were two dogs in which AF terminated after administering KCB 328 and one dog in which AF was terminated by dofetilide. KCB-328 and dofetilide were administered as a 2 min bolus followed by constant infusion per the protocol...
shown in Table 1. Propafenone was administered as a single bolus of 2 mg/kg followed by infusion at a rate of 0.07 mg/kg per min. We have previously shown this protocol provides stable propafenone levels (2.0–2.5 μg/ml) [10].

Doses of KCB-328 and dofetilide were increased until AF terminated or excess drug effect was manifested as either a QT interval \( \geq 15\% \) of control or premature ventricular depolarizations. When a therapeutic or proarrhythmic dose was established during an experiment on a given day, no further drug was administered. In the next experiment on the same animal, drug administration was started at two doses lower than the highest dose given previously and increased progressively until AF terminated or excess drug effect became evident. If any doses had not been administered by the last experimental day, they then were given at 10 min intervals as bolus without infusion. However, if drug toxicity supervened no further doses were given and the experiment was stopped.

AF and all other rhythms were monitored by continuous ECG recording. In dogs that did not convert to sinus rhythm after drug administration AF continued for hours to days. Electrical cardioversion was never used. Drug-induced AF conversion was considered successful when AF terminated within 2 h of any dose.

Blood samples for measuring KCB-328 and dofetilide levels were drawn at the following times: before administering any drug (at 0 min = control), at 2 min and 8 min after each dose, when AF converted to sinus rhythm, when excess QT prolongation or ventricular ectopy was observed, and at the end of the electrophysiological study. Plasma was separated, frozen and stored at \(-70^\circ\)C until analysed for drug levels as follows. KCB-328 was extracted from plasma samples with a liquid–liquid extraction column (EXtrelut® NT1, Merck KGaA, Germany): Plasma, 600 μl, was applied to the column for 10 min. The elution process was repeated three times and the eluted solvent was evaporated under a stream of nitrogen. The dry residue was suspended in 200 μl of acetonitrile (J.T. Baker, USA) (gradient A) and 0.1% trifluoroacetic acid (Sigma, USA) (gradient B). Finally, 30 μl of aliquot was injected into the High Performance Liquid Chromatography (HPLC). For the elution of plasma samples from dogs treated with dofetilide, only diethyl ether was used as solvent.

The concentrations of the compounds were assessed with an HP 1100 system (Hewlett-Packard GmbH, Germany) with a Hypersil BDS-C18, 125×2.0 mm I.D., 3 μm particle size column (Agilent, USA). Ultraviolet detection wavelength \( \lambda = 224 \pm 5 \) nm, and the flow rate \( = 0.25 \) ml/min. The linearity of the calibration curve was assessed from 10 to 1250 ng/ml.

**Electrophysiological methods**

All data were measured by an investigator who was blinded regarding the identity of the drug administered. Atrial ERPs were measured at the RAA and LAA at pacing cycle lengths (CL) of 400, 300 and 200 ms, and ventricular ERPs at the RV, LVA and RVOT at CL of 1000 and 400 ms. A single extra-stimulus was delivered at the end of a ten beat drive train, and ERP was defined as the shortest \( S_1-S_2 \) interval that produced a propagated response. All stimuli were given as 2 ms square waves at twice threshold current.

Local activation time was measured from the pacing artifact to the RAA and LAA electrograms during atrial pacing at CL = 400, 300 and 200 ms. Activation time was defined as the interval between the pacing spike and the maximum \( dv/dt \) of the electrogram. Atrial fibrillation CL was also measured from the RAA and LAA electrograms over 5 s and then averaged.

QT interval was calculated from the beginning of the Q wave to the end of the T wave. QRS duration
was similarly measured from the beginning of the Q wave to the end of the S wave, where it crossed the isoelectric line. All measurements are reported in milliseconds.

Data analysis

SPSS 10.0 software was used for statistical analysis. A paired \( t \)-test was used for paired data sets and Fisher’s exact test for incidence of events. Analysis of variance was used for repeated measures and data iteration performed for missing numbers. Incidence of AF correction by drug across groups was tested using chi square. Data are presented as means ± SEM. \( P < 0.05 \) was considered significant.

Results

Although eight doses of each drug were included in the KCB-328 and dofetilide protocols, only four dogs treated with KCB-328 and two with dofetilide received dose 8. In the other dogs excess QT prolongation or ventricular ectopy occurred at one of the earlier doses and the experiment was stopped. Hence, only data up to and including dose seven were used for statistical analysis. With respect to propafenone the drug was administered as a single bolus with infusion (see Section 2), so data were analyzed before and continually after drug administration.

Effects on atrial electrophysiology

Whereas KCB-328 and dofetilide dose-dependently and significantly increased AF CL measured in the RA (data not shown), only KCB-328 increased AF CL in the LA (Fig. 1). Propafenone increased AF CL in RA by 64 ± 9 ms and LA by 48 ± 8 ms (\( P < 0.05 \) for both). Termination of AF occurred in one of six dofetilide-treated dogs, three of six KCB-328 dogs, and eight of nine propafenone-treated dogs (\( P < 0.05 \)). Propafenone was more effective in terminating AF than dofetilide, but not different from KCB-328. ERP was measured in all dogs at control, after rapid atrial pacing (0–7 days before onset of AF), and again after drug administration in those animals in which AF terminated (Fig. 2). During the control period, RA ERPs were greater than LA. ERP shortened significantly in both chambers following rapid pacing: however, RA ERP remained greater than LA.

In those KCB328 or dofetilide treated dogs in which AF terminated, sinus rhythm returned 1–8 min after administering the effective dose. In three dogs in which AF terminated after administration of KCB-328, there was a non-significant change in RA ERP (15 ± 6 ms, \( P > 0.05 \)) and a 21 ± 5 ms decrease in the LA ERP only at CL 200 ms (\( P < 0.05 \)). There was no significant change in ERP at any other CL. In the single dofetilide-treated dog in which AF terminated, RA and LA ERPs were prolonged by 10–28 ms at all CL. In propafenone-treated animals in which AF terminated, sinus rhythm was seen 29 ± 7 min after drug was given. In these dogs, LA ERPs decreased from 96 ± 6 to 79 ± 3 ms (\( P < 0.05 \)) at CL = 400 ms. ERPs at other CL did not show a significant change.

Activation times were measured in the RA and LA at CL 400, 300 and 200 ms after rapid pacing and after treatment with KCB-328, propafenone or dofetilide. KCB-328 caused an increase in activation time in both atria (Fig. 3). This prolongation was significant in the RA at CL = 200 ms, and in the LA at CL = 400 ms (\( P < 0.05 \)). Propafenone prolonged activation time in both atria at all CL (\( P < 0.05 \)), as shown in Fig. 3. Activation time could be measured in only one dofetilide-treated animal in which AF terminated, and was unchanged compared with control.

Effects on ventricular electrophysiology

No change in QRS duration was seen with KCB-328 or dofetilide. For propafenone, QRS duration increased from 62 ± 3 to 70 ± 5 ms at 1000 ms (\( P < 0.05 \)) and from 68 ± 3 to 80 ± 3 ms at 400 ms (\( P < 0.05 \)).
A dose dependent prolongation of QT interval at pacing CL of 1000 ms was seen with both KCB-328 and dofetilide (Fig. 4) with the effect of dofetilide greater than that of KCB-328 at doses 4–7. We quantified the reverse use dependent effects of KCB-328 and dofetilide by calculating the magnitude of QT prolongation at CL 1000 and 400 ms and the percentage increase occurring over control. The increase in QT interval after KCB-328 was 40 ± 8 ms at CL 1000 ms, and 21 ± 4 ms at 400 ms (P < .05). However, the percentage changes of 15 ± 3% and 10 ± 2%, respectively, for the two cycle lengths did not differ (P > .05). In dofetilide-treated dogs there was a 47 ± 6 ms QT prolongation at CL 1000 ms compared with 20 ± 3 ms at 400 ms (P < .05). The percentage changes were 18 ± 2% and 9 ± 1% at CL 1000 and 400 ms, respectively (P < .05), indicating a greater reverse use dependent effect of dofetilide. QT interval changes with propafenone were small: the QT was 249 ± 6 ms at 1000 ms CL and 215 ± 9 at CL 400 ms before drug administration and 255 ± 5 (3 ± 1%) and 217 ± 8 ms (2 ± 1%) after the drug (both P > .05). No reverse use dependence was seen with propafenone.

KCB-328 increased the ventricular ERP from 180 ± 4 ms to 210 ± 7 ms at CL 1000 ms and from 149 ± 3 ms to 170 ± 4 ms at 400 ms (both P < .05). Dofetilide also prolonged the ventricular ERP at CL = 1000 ms (from 198 ± 9 ms to 228 ± 14 ms) and at CL 400 ms (from 157 ± 4 ms to 177 ± 5 ms), both P < .05. For propafenone, the ventricular ERP at CL 1000 ms was 182 ± 5 before and 187 ± 5 after propafenone administration (P > .05). At 400 ms, there was an increase from 153 ± 4 to 161 ± 5 (P < .05).

Ventricular premature depolarizations were seen in 4 of 6 animals treated with KCB-328 and 5 of 6 of those treated with dofetilide (P > .05). One of the six dofetilide-treated dogs manifested...
QT prolongation by 21% and subsequent polymorphic ventricular tachycardia and death. No mortality was seen in the KCB-328-treated dogs.

One propafenone-treated dog had premature ventricular depolarization and one had a short run of VT after bolus. No arrhythmias were seen in the remaining seven dogs. The incidence of arrhythmias after propafenone was less than after dofetilide ($P<.05$), and not statistically different from KCB-328.

**Plasma drug levels**

Fig. 5 shows the plasma levels of KCB-328 and dofetilide at 2 min after administering each bolus. Superimposed on the graphs are the concentrations at which therapeutic effects (prolongation of AF CL and termination of AF) and potential proarrhythmic effects (prolongation of the QT interval $>15\%$) occurred. Note that prolongation of AF CL and termination of AF occurred at concentrations lower than those inducing excess QT prolongation for KCB-328. The profile for dofetilide was less favourable in the sense that only AF CL was prolonged.

**Discussion**

The choice of therapy for atrial fibrillation is influenced by an assessment of whether the arrhythmia is paroxysmal (self-terminating), persistent (requiring cardioversion for termination) or permanent, and whether the goal is to return to and maintain sinus rhythm or to focus on ventricular rate control [2]. If atrial fibrillation is paroxysmal or persistent the question of pharmacological therapy becomes important. Some forms of drug treatment focus on the underlying disease process: for example, the administration of ACE inhibitors or angiotensin II receptor blockers to post-myocardial infarction patients and ACE inhibitors to congestive heart failure patients appears to delay the onset and reduce the incidence and recurrence of fibrillation [11,12]. Other forms of drug treatment focus on arrhythmogenic mechanisms per se: most such drugs are antiarrhythmics with actions to prolong repolarization and refractoriness (e.g. dofetilide, sotalol or amiodarone) and/or to slow conduction (e.g. flecainide, propafenone or procaine amide) [2]. In so doing these drugs can prevent/suppress reentry. However, a problem with all such drugs is the potential for proarrhythmia. For agents that
prolong repolarization and refractoriness excess QT interval prolongation may be a harbinger of the torsades de pointes that is the signature arrhythmia for acquired long-QT syndrome. Because of these problems the search continues for new drugs that might manifest antiarrhythmic efficacy while having less proarrhythmic potential.

It was for this reason that we studied KCB-328. This drug had previously been investigated in the experimental canine model of atrial flutter in which it prolonged atrial ERP and reduced dispersion of atrial refractoriness [13] slowed atrial conduction velocity, prolonged atrial flutter cycle length and terminated sustained flutter. The ventricular ERP and the QTc interval were prolonged but proarrhythmia did not occur. KCB-328 has shown potent antiarrhythmic effects in studies of canine ventricular arrhythmias induced by programmed electrical stimulation, and studies of ventricular fibrillation induced by coronary artery ligation [14].

In our study, KCB-328 significantly prolonged right and left atrial activation times and atrial fibrillation cycle length and terminated fibrillation in 3 of 6 dogs. Right atrial ERP and ventricular ERP and QT interval were also prolonged. In contrast, dofetilide terminated atrial fibrillation in one dog, while increasing atrial fibrillation cycle length but having no effect on atrial activation times. Ventricular ERP and QT interval were prolonged, and the reverse use-dependent effect on the QT interval was significant, the latter, in contrast to KCB-328. One death due to polymorphic ventricular tachycardia occurred with dofetilide; there was none with KCB-328. However, both drugs induced ventricular ectopy.

The mechanism of action of KCB-328, like that of the comparison methane sulphonamide compound, dofetilide, involves block of the Human ether-a-go-go related gene (HERG) channel resulting in the prolongation of action potential duration [15–17]. However, there are major differences in the interaction of the two drugs with their channel binding sites. Whereas both compounds bind preferentially to the activated state of IKr, use-dependently, only KCB-328 and not dofetilide, unbinds from the channel during the resting state [17,18]. This difference in action would be predicted to result in a lesser reverse use-dependency of KCB-328 effect on repolarization, as has been demonstrated in isolated guinea pig myocardium [6] and in the present study, in the intact dog. KCB-328 has no effect on other action potential parameters, including Vmax, action potential amplitude and resting membrane potential [6]. Hence, it is not quite clear why the drug would slow activation time in the atrium in our study, although such action may reflect an altered path length rather than a direct effect on excitability of the membrane or on the action potential upstroke. In addition, the effect on activation time is consistent with the slowing of atrial conduction induced by KCB-328 in the atrial flutter studies [13]. Finally, KCB-328 prolongs AF CL and atrial activation time while exhibiting less reverse use dependence in ventricle and less QT prolongation compared with dofetilide. Hence, it may be that the affinity of KCB-328 for atria prolongs is greater than that for dofetilide. However, this supposition would have to be tested.

We chose dofetilide as one reference compound for this study because it has been used clinically for conversion of atrial fibrillation to sinus rhythm and maintenance of the same. It is effective in patients with left ventricular dysfunction where its efficacy in terminating fibrillation was 59% compared with 34% with placebo [7]. Treatment has, however, been associated with adverse effects like QT prolongation, torsades de pointes and sudden death [8]. The comparison with KCB-328 in this study, while not showing a clear advantage of either drug with regard to termination of atrial fibrillation, pointed to the potential for a greater margin of safety with KCB-328. As shown in Fig. 5A, there is a clear demarcation between plasma levels of KCB-328 that prolong atrial fibrillation cycle length and induce significant (>15%) QT prolongation, the former effect occurring at under 100 ng/ml and the latter at 400 ng/ml. In the three animals in which atrial fibrillation was terminated, this occurred at approximately 200 ng/ml. In contrast, for dofetilide, atrial fibrillation cycle length prolongation occurred under 10 ng/ml and significant QT prolongation at 60 ng/ml, while AF termination occurred in one dog at 127 ng/ml.

We chose propafenone as the other reference compound because of its previously demonstrated success in treatment of atrial fibrillation [2]. In many instances propafenone or flecainide is used as first-line therapy here. In this particular model, propafenone had a significant effect on atrial fibrillation and was the only drug to do so. While this may reflect superior efficacy of this agent, it also may reflect the choice of model. Indeed, Wang et al. [19] have demonstrated the effectiveness of propafenone but not dofetilide in the pacing-induced atrial fibrillation model, whereas both drugs have a beneficial effect in fibrillation associated with congestive heart failure. With this in mind, one might anticipate a more salutary effect of KCB-328 in the congestive failure model.

In conclusion, the data presented here show that KCB-328 is an IKr blocker with less reverse
use-dependency than dofetilide. Accompanying this action appears to be a lower proarrhythmic potential, suggesting the drug warrants further study as an alternative therapy for termination/prevention of atrial fibrillation.

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