Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 486 (2004) 209–221
www.elsevier.com/locate/ejphar

Differential effect of HERG blocking agents on cardiac electrical alternans in the guinea pig

Anthony A. Fossa*, Todd Wisialowski, Eric Wolfgang, Ellen Wang, Michael Avery, David L. Raunig, Bernard Fermini

Pfizer Global Research and Development, Eastern Point Rd., Bldg. 118, MS 4036 Groton, CT 06340, USA

Received 22 September 2003; accepted 19 December 2003

Abstract

Beat-to-beat alternations of the cardiac monophasic action potential, known as electrical alternans, were studied at drug concentrations that have known arrhythmogenic outcomes. Electrical alternans were elicited from the heart of anesthetized guinea pigs, both in the absence and presence of drugs that inhibit the delayed rectifier K^+ channel encoded by the *human ether a-go-go related-gene* (HERG), and are associated with the fatal arrhythmia, Torsade de Pointes. Two other HERG inhibiting drugs not associated with Torsade de Pointes were also studied. At concentrations known to be proarrhythmic, E-4031 and bepridil increased mean alternans 10 and 40 ms at pacing frequencies ≤ 160 ms. Terfenadine and cisapride both increased mean alternans up to 20 and 21 ms, respectively, at pacing frequencies of ≤ 150 ms. On the other hand, verapamil and risperidone showed no increase in mean alternans while risperidone significantly reduced alternans at concentrations up to 74 times its therapeutic level. The magnitude of effect on rate-dependent alternans may allow the differentiation of proarrhythmia and non-arrhythmic HERG blockers at clinically relevant concentrations.

Keywords: Alternans; HERG; Torsade de Pointes; QT prolongation; Arrhythmia

1. Introduction

Over the last decade, several non-cardiac drugs that affect repolarization of the heart have been withdrawn from the marketplace because of their propensity to lengthen the QT interval on the surface electrocardiogram and increase the incidence of developing Torsade de Pointes, a potentially fatal polymorphous ventricular arrhythmia. To date, all commercial compounds known to cause Torsade de Pointes have been shown to prolong the QT interval and preferentially inhibit the delayed rectifier K^+ channel encoded by the *human ether a-go-go related-gene* (HERG) that can be expressed in a variety of cell lines, and believed to represent the alpha-subunit of I_{Kr} channels. Prolongation of the QT interval is the only clinical surrogate marker currently used by worldwide regulatory agencies to assess the risk of developing this arrhythmia. Electrophysiological assays, and more specifically the whole-cell voltage-clamp technique, are extensively used in the pharmaceutical industry to study the effects of new chemical entities on the HERG channel. However, it is now becoming apparent that in many cases, drugs that inhibit HERG current and/or prolong the QT interval are not associated with the occurrence of Torsade de Pointes. Therefore, additional information is necessary to determine if a compound that inhibits HERG current has the propensity to be proarrhythmic. Although several different in vitro (Yan and Antzelevitch, 1998; Hondeghem et al., 2001) and in vivo models (De Clerck et al., 2002) are currently available to help identify the potential for arrhythmia, their ability to quantitatively predict the clinical outcome at therapeutic concentrations is often limited. Early identification of the risk of new chemical entities or their metabolites, to induce QT prolongation has become an integral part of the development process of every new drug, and there is an urgent need to develop surrogate markers that will predict more accurately the proarrhythmic potential and clinical outcomes of drugs.

The action potential duration (APD) restitution property of the heart is characterized by the relationship of the APD accompanied by the preceding diastolic interval

* Corresponding author. Tel.: +1-860-441-4094; fax: +1-860-715-7636.
E-mail address: Anthony_A_Fossa@groton.pfizer.com (A.A. Fossa).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.12.028

(Walker and Rosenbaum, 2003). Electrical alternans of the action potential develops when a series of short–long diastolic interval cycles generates a reciprocating long–short series of APD. The onset and degree of alternans are heart rate-dependent (Franz et al., 1988; Koller et al., 1998), and when large enough can result in conduction block that may transition the heart from a synchronous stable ventricular tachycardia to fibrillation (Rosenbaum et al., 1994). As a result, it has been suggested that electrical alternans may be a precursor to the development of ventricular arrhythmias (Gilmour and Chialvo, 1999) when waveform breakup occurs (Chen et al., 1997). Assessment of the restitution curve across a range of heart rates to determine a restitution slope using several protocols has revealed an inconsistent pattern of prediction for cardiac drugs known to cause arrhythmias because the contribution of cardiac memory/accommodation is not accounted for (Koller et al., 1998; Gilmour et al., 1997). Accommodation may be especially important when trying to understand why patients with either acquired or congenital long QT syndromes exhibit greater sensitivity toward arrhythmia when changes in heart rate occur suddenly after a startle, emotional event or exercise (Schwartz et al., 2001).

In the present study, we have used APD changes and the occurrence of electrical alternans measured on a beat-to-beat basis with increasing pacing frequency to predict the proarrhythmic potential of both cardiac and non-cardiac HERG blocking drugs used at clinically relevant concentrations. We also determined the potency of these drugs on HERG and L-type Ca^{2+} currents at similar concentrations. The proarrhythmic drugs used were E-4031 (class III antiarrhythmic), bepridil (Ca^{2+} channel blocker), terfenadine (antihistamine) and cisapride (gastrointestinal prokinetic agent). These drugs are all associated with reported cases of Torsade de Pointes. We also studied the effects of verapamil (Ca^{2+} channel blocker) and risperidone (antipsychotic), two drugs that are potent inhibitors of HERG, but associated with a low incidence of Torsade de Pointes. The data suggests that alternans may possibly be used as a preclinical biomarker to predict the clinical outcome of compounds and differentiate between safe and proarrhythmic HERG blockers.

2. Materials and methods

2.1. Drugs

2.1.1. Voltage-clamp experiments and in vivo experiments

E-4031 (1-[2(6-methyl-2-pyridyl)ethyl]-4-(4-methylsulfonylamidobenzoyl)piperidine) and cisapride were synthesized for use by the Department of Chemistry at Pfizer (Sandwich, Kent, UK or Groton, CT). Bepridil, terfenadine and (±)-verapamil were purchased from Sigma (St. Louis, MO). Risperidone was purchased from both Sigma and ICN Biomedicals (Aurora, OH). All drugs were prepared as a 10 mM stock solution in dimethyl sulfoxide for voltage-clamp experiments except E-4031 that was prepared as a 1 mM stock in water. All drugs were dissolved in 30% sulfobutylether cyclodextrin in distilled water except for E-4031 (saline used) for in vivo studies. Vehicle in vivo studies used 30% sulfobutylether cyclodextrin in distilled water or saline administered separately.

2.2. Whole-cell voltage clamp

The human embryonic kidney cell line 293 (HEK293) expressing HERG, as well as recording solutions and data analysis used in the present studies were described previously (Volberg et al., 2002).

2.2.1. Isolation of cardiac myocytes and recording solutions for Ca^{2+} currents

Male guinea pigs weighing approximately 350 g were anesthetized with isoflurane. The hearts were quickly removed, mounted via the aorta to a Langendorff apparatus, and perfused with 37 °C oxygenated Buffer A (described below). After 3–5 min of perfusion the buffer was changed to one containing collagenase (0.5 mg/ml, Worthington Biochemical, Lakewood, NJ), protease (0.033 mg/ml, Sigma type XIV), and fetal bovine serum (0.5 μl/ml) for 10–15 min. Then the ventricles were removed from the apparatus, chopped with scissors into pieces and further digested in the enzyme-containing solution at 37 °C for 3 min with a gentle shaking. The cell suspension was filtered through a 180-μm nylon mesh and centrifuged at 500–600 rpm for 5 min. The supernatant was removed and the pellet was resuspended with enzyme-free Buffer A. The Ca^{2+} concentrations were gradually restored to a final concentration of 1 mM (from 0.25 to 0.5, 0.75 and 1.0 mM). Cells were finally stored in 1.0 mM Ca^{2+}-containing Buffer A at room temperature for at least 1 h prior to use.

2.2.2. Solutions for Ca^{2+} currents

Buffer A was composed of (in mM): NaCl, 133; KCl, 4.4; $MgSO_4$, 1.2; glucose, 11; $NaHCO_3$, 4; 2,3-butanedione monoxime (BDM), 15; HEPES, 10; pH 7.35 with NaOH. The bath solution used to record the Ca^{2+} currents had the following ionic composition of (in mM): Tris, 137; $CaCl_2$, 1.8; $MgCl_2$, 1; glucose, 5; CsCl, 20; pH 7.4 with CsOH. The internal pipette solution was composed of (in mM): CsCl, 125; MgATP, 5; HEPES, 10; EGTA 15, TEA–Cl, 20; pH 7.2 with CsOH.

2.2.3. Data acquisition and analysis

Ca^{2+} current experiments were performed at room temperature (20–22 °C). Currents were measured using the whole-cell configuration of the patch clamp technique. Recordings were started 10 min after membrane rupture to allow cell dialysis with the pipette solution. Cells were held

at −70 mV. L-type Ca^{2+} current was elicited by a 250-ms depolarization to 10 mV following a 200-ms prepulse at −40 mV to inactivate the T-type Ca^{2+} current. Holding the cells at −70 mV was helpful to slow the "rundown" process of this current. After several minutes the current stabilized into a rundown that was well described by a linear regression. To accurately determine the potency, the control current amplitude was calculated by applying a linear regression through the linear portion of the control data.

2.3. Monophasic action potential duration (MAPD) alternans measurements in the anesthetized guinea pig

2.3.1. Surgical procedure

Animal care conforms to US National Institutes of Health (NIH Publication No. 8523, revised 1996) and European guidelines for use of experimental animals. All protocols were approved by the Pfizer Institutional Animal Care and Use Committee. Male Hartley guinea pigs weighing 400–800 g were anesthetized with Nembutal 40 mg/kg, ip. Supplemental anesthetic was given if necessary and the guinea pigs were placed on a heating pad for the duration of the surgery and experiment. A tracheotomy was performed and the guinea pigs were ventilated with room air using a Harvard Apparatus Rodent Ventilator Model 683 (South Natick, MA). The right jugular vein and left carotid artery were cannulated with PE50 tubing for drug administration and blood pressure monitoring, respectively. A medial thoracotomy was performed allowing the heart to be suspended in a pericardial cradle. The heart was frequently moistened with lactated Ringer's solution. Two Teflon-coated silver wires (0.013 in. diameter; A-M Systems, Carlsborg, WA) were sutured to the left ventricular apex for pacing with a Bloom Technologies DTU 215 Programmable Stimulator (Fischer Imaging, Denver CO). Monophasic action potential (MAP) signals were recorded from the left ventricle with an EP Technologies EPT 200 Probe (San Jose, CA) that was held in position with a modified stereotaxic stand. Once the MAP signal was stable, the probe remained fixed in the same position throughout the entire study. Lead II electrocardiogram (ECG) signals were recorded with 22-gauge needle electrodes placed subcutaneously around the thoracotomy. The MAP, pacing stimulus, ECG and blood pressure waveforms were sampled at 1000 Hz and saved to disk using a Po-Ne-Mah data acquisition and analysis system (Gould, Valley View, OH).

2.3.2. Dosing protocol

Guinea pigs ($n=6$ each drug and vehicle) received vehicle or drug intravenously using the dose escalation protocol described in Table 1. This protocol was designed to establish a dose–response relationship for changes in the measured parameters and to attain plasma concentrations similar to those achieved clinically. Drugs or an equal volume of their respective vehicle were administered as a initial loading dose over 5 min followed by a maintenance infusion for 10 min to reach each of four steady plateau drug concentrations during the period of data collection. All solutions were infused with a PHD 2000 Programmable Pump (Harvard Apparatus). Separate pharmacokinetic studies were performed in anesthetized guinea pigs in which arterial blood samples were taken at the end of each plateau phase during the prescribed iv infusion protocol.

Table 1
Dose escalation protocol for the various drugs administered intravenously in the anesthetized guinea pig

Drug	iv loading (μg/kg)	iv maintenance (μg/kg/h)	Total dose (μg/kg)
E-4031	8.0	2.9	8.5
	24.0	11.5	25.9
	96.0	46.1	103.5
	384.0	184.3	414.7
Bepridil	256.0	325.6	310.3
	512.0	1302.6	729.1
	1024.0	5210.2	1892.4
	2048.0	20841.0	5521.5
Terfenadine	40.0	64.0	50.7
	120.0	256.0	162.7
	480.0	1024.0	650.7
	1920.0	4096.0	2602.7
Cisapride	40.0	12.8	42.1
	120.0	51.2	128.5
	480.0	204.8	514.1
	1920.0	819.2	2056.5
Verapamil	2.0	1.3	2.2
	8.0	5.1	8.8
	32.0	20.4	35.4
	128.0	81.4	141.6
Risperidone	40.0	32.4	45.4
	160.0	129.6	181.6
	640.0	518.4	726.4
	2560.0	2073.6	2905.6

2.3.3. Pacing protocol

MAP signals (>15 mV) were filtered 0.05–1000 Hz and allowed to stabilize for at least 10 min before the pacing protocol. The minimum threshold current for ventricular capture was determined (0.1–0.4 mA). Pacing was performed at twice threshold current. A preconditioned pacing protocol, similar to a dynamic restitution pacing protocol, was applied as follows: a 50-beat preconditioning pulse train (Basic Cycle Length (BCL) S1 = 220 ms or S1 = 240 ms) was followed immediately by a 30-beat pulse train at BCL S2 = 200 ms. After a small pause to adjust the stimulator (5–15 s), pacing was reinitiated at the 50-beat preconditioning S1 pulse train followed by a new 30-beat pulse train at S2 = 190 ms. This protocol was repeated with a fixed S1 cycle length and from S2 = 200 ms to S2 = 140 ms by 10 ms decrements, until an arrhythmia (Torsade de Pointes-like) occurred or the heart became refractory. This pacing protocol was repeated twice during baseline to ensure stable control conditions and during the last 5 min

of each maintenance infusion. The total duration of the experiment with pacing was approximately 90 min.

2.3.4. MAPD measurements

MAPD measurements were automatically performed by the Po-Ne-Mah system and defined as the time from the maximum rate of depolarization (V_{max}) to 90%, 50% and 30% repolarization. Signals recorded during each study were replayed on the Po-Ne-Mah system and the MAP signals were visually inspected to verify ventricular capture of the stimulus and to validate measurements during the pacing protocol. If necessary, manual measurements were made using a digital measure command on the Po-Ne-Mah.

MAP signals that were captured and measured during S2 pacing were used for alternans analysis. The first two pulsed beats at each cycle length were removed to account for possible incomplete capture. Beat-to-beat alternans were calculated as the absolute difference between two consecutive MAPD measured at 50% repolarization ($MAPD_{50}$) and averaged over the entire S2 pulse train to yield an average value. In order to avoid interpolation errors at the end of repolarization when alternans develops at faster pacing cycles and still account for Phase III of the MAPD signal, the $MAPD_{50}$ was used for mean alternans calculation rather than $MAPD_{30}$ or $MAPD_{90}$. Data were included for a given study only if the difference between the two baseline periods indicated a stable experimental preparation; 5 ms BCL 200–170, 10 ms at 160, and 15 ms at 150–140. If these criteria were met, data for each study was calculated as the difference from the second baseline period.

2.3.5. Analytical procedure

Guinea pig plasma was purchased from Harlan Bioproducts (Indianapolis, IN) and protein binding studies were carried out at the following concentrations: 0.5 μg/ml for cisapride, 0.1 μg/ml for risperidone, 0.5 μg/ml for terfenadine, 0.05 and 0.5 μg/ml for verapamil, and 2, 6 and 10 μg/ml for bepridil. Plasma protein binding studies were conducted using a Spectra/Por equilibrium dialysis system (Spectrum, Rancho Dominguez, CA) as described by Reed-Hagen et al. (1999). The unbound fraction (f_u) of drug in plasma was calculated according to Boudinot and Jusko (1984). E-4031 plasma protein binding was determined in guinea pig plasma using the same methods as reported by Webster et al. (2001).

2.4. Analysis of drug in plasma

A method involving solid phase extraction and High Performance Liquid Chromotography/Mass Spectrometry in a multiple reaction-monitoring mode (HPLC/MS/MS) for analysis was used to determine drug concentration in all samples. Briefly, 50 μl of sample along with the internal standard (disopyramide) were extracted using a Waters Oasis MCX extraction plate (Waters, Milford, MA) and eluted twice with 200 μl of methanol/ammonium hydroxide (95:5). The eluent was evaporated to dryness under nitrogen, and the residue was reconstituted in 50–200 μl of mobile phase (40% of 10 mM ammonium acetate with 0.05% formic acid/60% of acetonitrile with 0.05% formic acid). Reconstituted extracts were injected (10–20 μl) onto a cyano column (2 × 50 mm, 5 μm particle size) at a flow rate of 0.2–0.25 ml/min and detected using an API-4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Foster City, CA).

2.5. Expression of results and statistical analyses

SAS Version 8 (SAS Institute, Cary, NC) was used in the analyses of average MAPD alternans, heart rate and blood pressure. The experiment used a completely randomized design. The data were analyzed as mixed effects, repeated

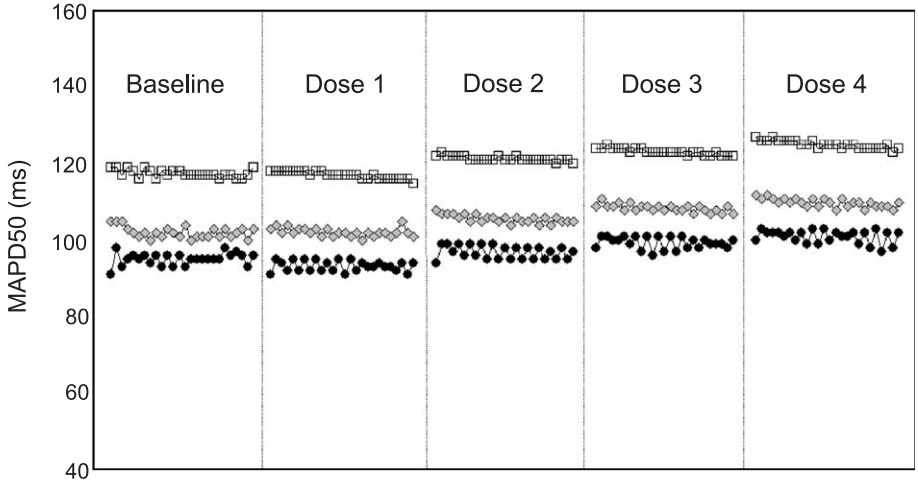

Fig. 1. Individual beat-to-beat $MAPD_{50}$ after 30 successive pulses at BCL-200 (□), BCL-170 (◆) and BCL-150 (●) before (baseline) and during 30% sulfobutylether cyclodextrin vehicle infusion.

measures model using the Mixed procedure in SAS. The covariance structure, compound symmetry, was chosen using the optimum Akaike Information Criteria. Comparisons were done in the Mixed procedure to determine differences from baseline and compare them to the respective vehicle differences. Changes in blood pressure, heart rate, $MAPD_{90}$, $MAPD_{50}$, and mean alternans are represented as difference from vehicle response. A contrast was used for each drug to simultaneously determine significance over all the dosing periods. Following a significant overall contrast, individual contrasts were used to determine the significant differences at each dosing period. This allowed for specific planned comparisons to be done using the error information from all the data. Multiple comparisons used Fisher's Protected Least Significant Difference method to preserve the experiment-wise error rate. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Effect of vehicle (sulfobutylether cyclodextrin or saline) on electrical alternans

Effects observed with sulfobutylether cyclodextrin or saline vehicles were similar. Resting heart rate declined from -7 to -13 beats per minute (BPM) while blood pressure remained unchanged. $MAPD_{50}$ and $MAPD_{90}$ at a basic cycle length of 200 ms (BCL-200) increased slightly (9 and 7 ms, respectively). The mean alternans duration did not change when paced from BCL-200 to 150 ms. An increase in alternans of 15 ± 10 ms occurred at BCL-140 for saline and 14 ± 11 ms for sulfobutylether cyclodextrin vehicles. A typical example of beat-to-beat $MAPD_{50}$ for varying cycle lengths is shown in Fig. 1. Note that alternans begins to increase at BCL-150.

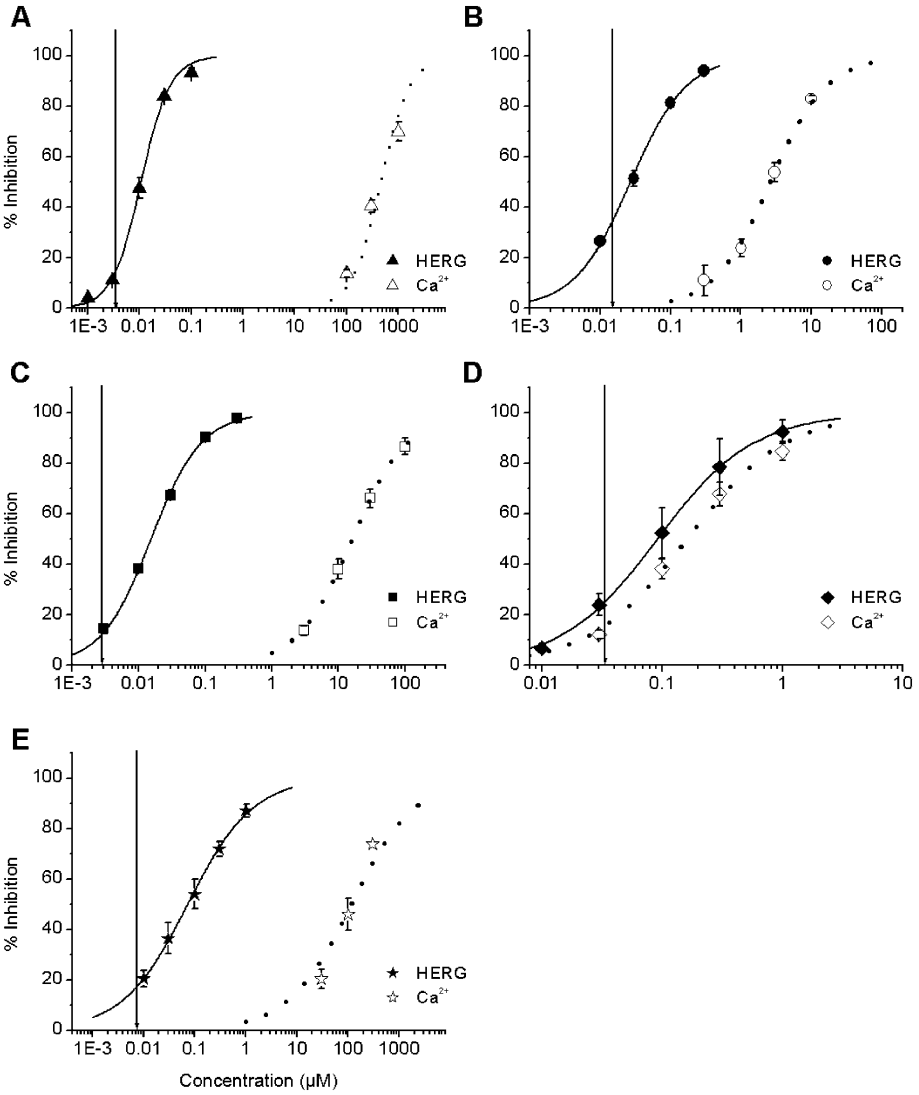

Fig. 2. Overlay plots of dose–response relationships for HERG and $I_{Ca\text{-}L}$ for E-4031 (A), bepridil (B), cisapride (C), verapamil (D) and risperidone (E). The vertical line indicates the effective therapeutic concentration (unbound) in humans.

3.2. Effect of drugs associated with Torsade de Pointes on electrical alternans

3.2.1. E-4031

E-4031 inhibited HERG K^+ current with an IC_{50} of approximately 11 nM (Fig. 2) and had little effect on Ca^{2+} currents (IC_{50} = 441 μM). The IC_{20} value of HERG inhibition by E-4031 (5 nM) was close to the clinically relevant human therapeutic unbound concentration of 3.3 nM (4.3 ng/ml total; Fujiki et al., 1994). E-4031 reduced heart rate, but not blood pressure, in a dose-dependent manner approximately −35 to −57 BPM beyond the vehicle response beginning at 5.3 up to 97.3 nM. At the lower concentration of 5.3 nM E-4031 produced a statistically significant increase of 12 ms in $MAPD_{50}$ and 15 ms in $MAPD_{90}$ at BCL-200 (Table 2). At this cycle length, increases of up to 17 and 19 ms in $MAPD_{50}$ and $MAPD_{90}$ were observed at 25.2 nM, with no further increases apparent at 97.3 nM. The mean alternans were significantly increased at shorter cycle lengths of (≤ BCL-160). The largest magnitude of alternans change was 17 ms at BCL-150 at concentrations ranging from 5.34 to 97.3 nM (Fig. 3). At 97.3 nM and cycle lengths ≤ BCL-160, pacing was not possible in many of the animals because of either refractoriness or generation of a Torsade de Pointes-like arrhythmia. This also occurred in a concentration-related manner at the lower concentrations but at shorter pacing cycles. In addition, animals paced at these shorter cycles and exposed to higher plasma concentrations developed a discordant pattern of beat-to-beat alternans (Fig. 4).

3.2.2. Bepridil

Bepridil is a Ca^{2+} channel blocker (Ca^{2+} IC_{50} = 2600 nM) that shows a higher potency for HERG over $I_{Ca\text{-}L}$ (Fig. 2, HERG IC_{50} = 26 nM). The therapeutic unbound concen-

Table 2
Hemodynamic parameters measured at sinus rhythm (heart rate and blood pressure) and monophasic action potential duration measured at BCL-200 (MAPD50 and MAPD90) during vehicle or drug infusion in the anesthetized guinea pig

Treatment		Baseline	Step 1	Step 2	Step 3	Step 4
Vehicle	Heart rate	226 ± 7	219 ± 7	215 ± 6	213 ± 9	213 ± 10
	Blood pressure	43 ± 3	44 ± 3	44 ± 3	42 ± 3	42 ± 2
	$MAPD50_{(200)}$	118 ± 3	121 ± 2	125 ± 2	127 ± 2	127 ± 2
	$MAPD90_{(200)}$	137 ± 2	139 ± 2	143 ± 2	144 ± 2	143 ± 2
E-4031, MW = 402, f_u = 35.6%	Heart rate	227 ± 7	195 ± 4	181 ± 6[a]	170 ± 7[a]	157 ± 15[a]
	Blood pressure	39 ± 1	39 ± 1	40 ± 1	43 ± 1	43 ± 2
	$MAPD50_{(200)}$	112 ± 2	122 ± 2[a]	131 ± 2[a]	136 ± 2[a]	138 ± 3[a]
	$MAPD90_{(200)}$	135 ± 1	145 ± 2[a]	154 ± 2[a]	158 ± 2[a]	158 ± 3[a]
	Free drug, nM		1.28 ± 0.16	5.34 ± 1.3	25.2 ± 7.0	97.3 ± 25.7
Bepridil, MW = 366, f_u = 0.0119–0.0471%	Heart rate	212 ± 8	201 ± 5	186 ± 2	165 ± 5[a]	140 ± 4[a]
	Blood pressure	44 ± 2	46 ± 2	45 ± 2	44 ± 1	38 ± 1
	$MAPD50_{(200)}$	117 ± 3	123 ± 4	132 ± 3[a]	138 ± 3[a]	135 ± 3[a]
	$MAPD90_{(200)}$	138 ± 2	144 ± 3	152 ± 3[a]	160 ± 3[a]	163 ± 3[a]
	Free drug, nM		0.0558 ± 0.01	0.448 ± 0.185	2.66 ± 0.48	11.1 ± 3.1
Terfenadine, MW = 472, f_u = 0.035	Heart rate	226 ± 5	224 ± 5	211 ± 7	199 ± 6	172 ± 8[a]
	Blood pressure	43 ± 3	45 ± 3	43 ± 2	43 ± 1	36 ± 2
	$MAPD50_{(200)}$	118 ± 1	120 ± 2	128 ± 3	135 ± 3[a]	142 ± 2[a]
	$MAPD90_{(200)}$	138 ± 2	140 ± 2	148 ± 3	154 ± 2[a]	163 ± 2[a]
	Free drug, nM		0.79 ± 0.08	2.68 ± 0.40	10.0 ± 0.81	45.8 ± 7.85
Cisapride, MW = 466, f_u = 0.133	Heart rate	216 ± 6	196 ± 6	178 ± 7[a]	163 ± 7[a]	138 ± 4[a]
	Blood pressure	42 ± 4	42 ± 3	40 ± 3	41 ± 4	37 ± 4
	$MAPD50_{(200)}$	118 ± 4	124 ± 3	129 ± 3	134 ± 3[a]	137 ± 2[a]
	$MAPD90_{(200)}$	139 ± 3	146 ± 3[a]	151 ± 3[a]	155 ± 3[a]	157 ± 3[a]
	Free drug, nM		1.89 ± 0.26	6.42 ± 0.53	23.3 ± 2.62	114 ± 21.3
Verapamil, MW = 454, f_u = 4.68%	Heart rate	208 ± 6	205 ± 5	202 ± 5	195 ± 5	181 ± 7
	Blood pressure	36 ± 5	36 ± 4	35 ± 4	36 ± 4	33 ± 4
	$MAPD50_{(200)}$	119 ± 1	120 ± 2	124 ± 3	124 ± 3	126 ± 2
	$MAPD90_{(200)}$	139 ± 1	140 ± 1	142 ± 2	142 ± 2	144 ± 2
	Free drug, nM		0.288 ± 0.05	0.935 ± 0.098	3.39 ± 0.72	13.2 ± 4.5
Risperidone, MW = 410, f_u = 0.164	Heart rate	216 ± 4	229 ± 9	224 ± 9	233 ± 11	227 ± 5
	Blood pressure	40 ± 2	33 ± 2	35 ± 2	33 ± 1	28 ± 2
	$MAPD50_{(200)}$	113 ± 3	108 ± 4	100 ± 1	93 ± 1	94 ± 2
	$MAPD90_{(200)}$	136 ± 2	133 ± 3	121 ± 1	120 ± 2	121 ± 2
	Free drug, nM		6.1 ± 0.58	29.6 ± 3.13	131 ± 12.1	591 ± 42.0

Refer to Table 1 for exact doses used for each step of the escalation protocol. Vehicle was administered in the same dose volume used for the various standards. All values were calculated using the Mixed procedure in SAS V6.12 and represent the least squares mean ± S.E. (n = 4–7).

[a] Denotes significant difference from pre-dose baseline compared to respective vehicle difference from pre-dose baseline at each dose level within a given treatment, $P < 0.05$.

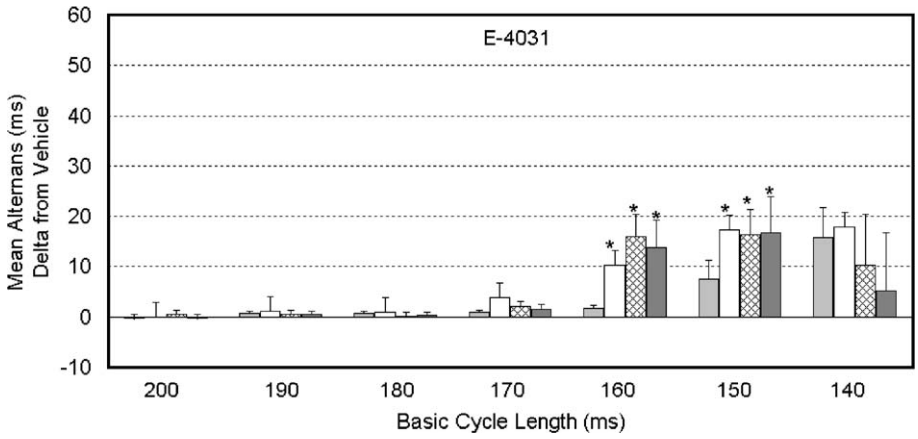

Fig. 3. Mean alternans of beat-to-beat $MAPD_{50}$ in anesthetized guinea pigs ($n=3-6$) treated with E-4031 by intravenous infusion at free drug plasma concentrations of 1.28 ■, 5.34 □, 25.2 ☒ and 97.3 nM). *Denotes significant difference from pre-dose baseline compared to respective vehicle difference from pre-dose baseline at each dose level within a given treatment, $P<0.05$.

tration of bepridil is approximately 15 nM (2386 ng/ml total drug; Lesko et al., 1986; Benet, 1985). In vivo, this compound produced a dose-related decrease in heart rate, but not blood pressure, with a maximal effect of − 59 BPM occurring at the highest concentration of 11.1 nM (Table 2). When paced at BCL-200, bepridil significantly increased $MAPD_{50}$ and $MAPD_{90}$ by 8 ms at a concentration of 0.45 nM. Higher concentrations of bepridil increased $MAPD_{50}$ by 12 ms at 2.7 nM and $MAPD_{90}$ by 19 ms at 11.1 nM. The maximal concentration of bepridil produced rate-dependent increases in mean alternans ranging from 13 ms at BCL-200 to 40 ms at BCL-160 (Fig. 5). The increase in mean

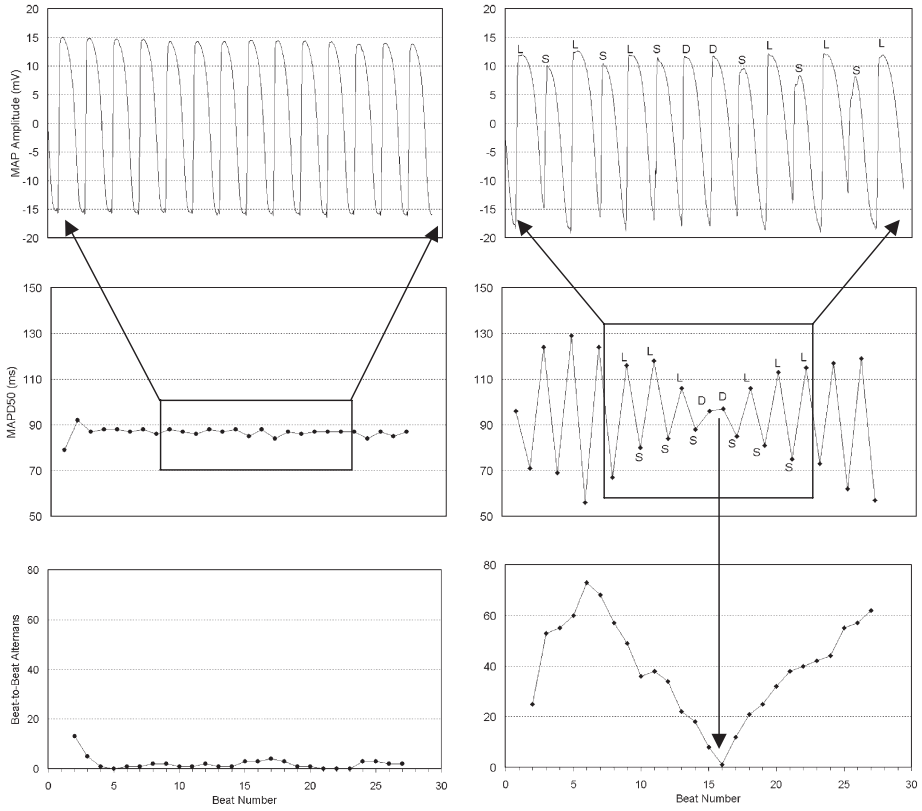

Fig. 4. Individual beat-to-beat MAPD tracings, $MAPD_{50}$ and magnitude of alternans within a 30 successive pulse train at BCL-150 during saline vehicle (1A–3A) or E-4031 (1B–3B) infusion at a free plasma concentration of 25.2 nM. Note transition from concordant long (L) and short (S) MAPD to (D) discordant alternans cycles with E-4031.

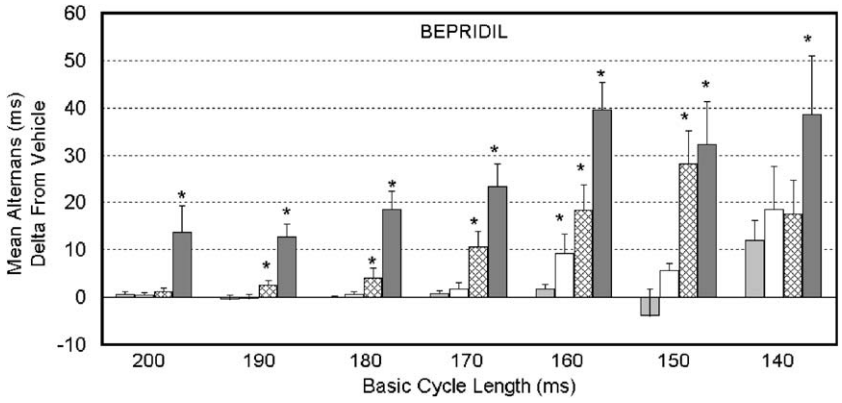

Fig. 5. Mean alternans of beat-to-beat $MAPD_{50}$ in anesthetized guinea pigs ($n=3-6$) treated with bepridil by intravenous infusion at free drug plasma concentrations of 0.0558 ■, 0.448 □, 2.66 ☒ and 11.1 nM). *Denotes significant difference from pre-dose baseline compared to respective vehicle difference from pre-dose baseline at each dose level within a given treatment, $P<0.05$.

alternans was also statistically significant at lower concentrations of 2.7 nM at BCL-190 and 0.45 nM at BCL-160.

3.2.3. Terfenadine

Terfenadine inhibited HERG K^+ current with an IC_{50} of 11 nM, with some effect on L-type Ca^{2+} channel currents (IC_{50} estimated at around 250 nM), although the inhibition of Ca^{2+} currents could not be determined accurately at low concentrations because of the slow onset of block. The IC_{20} value for HERG inhibition (2.5 nM) was within the clinically relevant human therapeutic unbound concentration range of 0.6–3.8 nM (8–50 ng/ml total; McTavish et al., 1990; Honig et al., 1993). In anesthetized guinea pigs, a concentration-related decrease in sinus heart rate, but not blood pressure, was observed at or above 3 nM that became statistically significant at 46 nM (Table 2). Mean alternans increased in a rate- and concentration-related manner (Fig. 6). A 6 ms increase occurred at BCL-140 at 0.8 nM free drug, 16–20 ms increases occurred at BCL-150 and BCL-140 at 10 nM, and at all cycle lengths (BCL-200 to BCL-140) at 46 nM (4–32 ms). Significant increases in $MAPD_{50}$ and $MAPD_{90}$ at BCL-200 were observed at all drug concentrations of 10 nM or more even though alternans were not apparent at this longer cycle length.

3.2.4. Cisapride

Cisapride was a potent inhibitor of HERG current ($IC_{50}=15$ nM, Fig. 2) but showed little effect on L-type Ca^{2+} channel ($IC_{50}=16{,}000$ nM) currents. The therapeutic free drug concentration of cisapride ranges from 2.7 nM (50 ng/ml total; Gladziwa et al., 1991) in most patients to approximately 6 nM in the presence of a cytochrome *P*-450 3A4 (CYP-3A4) metabolic inhibitor (van Haarst et al., 1998). In the anesthetized guinea pig, there was very little change in the basal heart rate and blood pressure at 1.9 nM but a dose-related decrease in heart rate from −27 to −65 BPM was observed from 6.4 to 114 nM (Table 2). Mean alternans was more affected by pacing rate than concentration (Fig. 7). A 9 ms increase occurred at BCL-140 at the 1.9 nM dose level. At the 6.4 and 23 nM free drug levels, 5 and 21 ms increases, respectively, were observed at BCL-150. No statistically significant changes were observed at

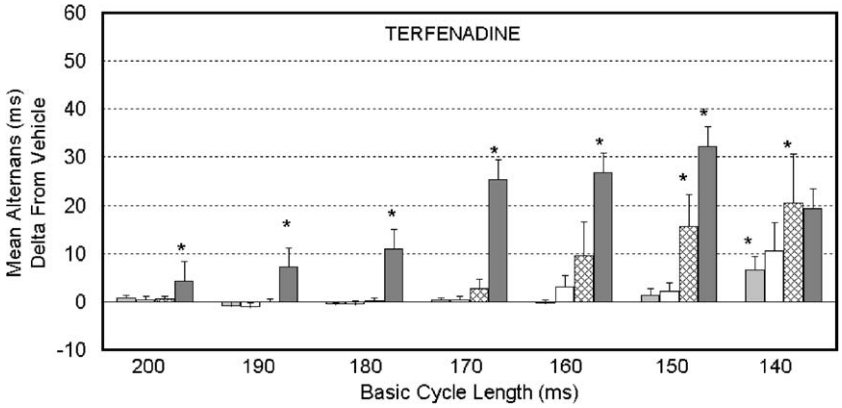

Fig. 6. Mean alternans of beat-to-beat $MAPD_{50}$ in anesthetized guinea pigs ($n=3-6$) treated with terfenadine by intravenous infusion at free drug plasma concentrations of 0.79 ■, 2.68 □, 10.0 ☒ and 45.8 nM). *Denotes significant difference from pre-dose baseline compared to respective vehicle difference from pre-dose baseline at each dose level within a given treatment, $P<0.05$.

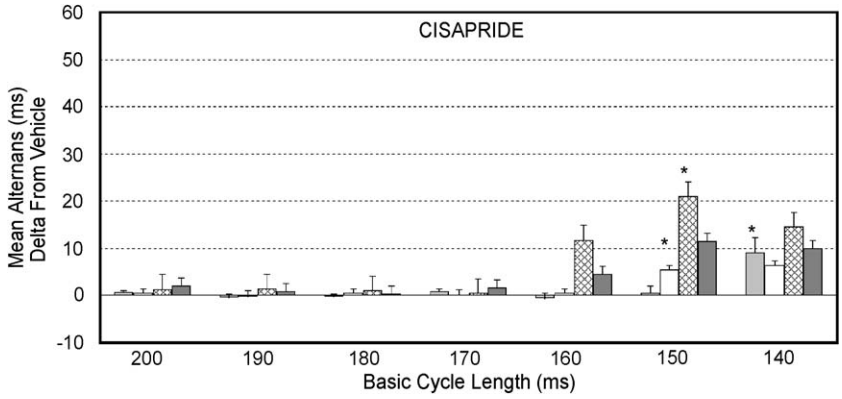

Fig. 7. Mean alternans of beat-to-beat $MAPD_{50}$ in anesthetized guinea pigs ($n=3-6$) treated with cisapride by intravenous infusion at free drug plasma concentrations of 1.89 ■, 6.42 □, 23.3 ☒ and 114 nM). *Denotes significant difference from pre-dose baseline compared to respective vehicle difference from pre-dose baseline at each dose level within a given treatment, $P<0.05$.

114 nM because of the large variability in the responses, and three of the six animals studied at this concentration did not meet the requirement for inclusion of pacing cycles at BCL-140. $MAPD_{50}$ and $MAPD_{90}$ began to increase at the clinically relevant concentration of 1.9 nM, but no changes were observed in alternans.

3.3. Effect of drugs not associated with Torsade de Pointes on electrical alternans

3.3.1. Verapamil

Like bepridil, verapamil is a Ca^{2+} channel blocker that also inhibits HERG currents (Fig. 2, HERG $IC_{50}=94$ nM; Ca^{2+} $IC_{50}=164$ nM). However, at a free therapeutic concentration of approximately 34 nM (154 ng/ml total; Muller et al., 1986), verapamil inhibits both currents with similar potency, in sharp contrast with bepridil (see discussion). No statistically significant changes were observed in heart rate, blood pressure, $MAPD_{50}$ or $MAPD_{90}$ (Table 2) at concentrations up to 13.2 nM. Alternans were no greater with verapamil than with vehicle control and appeared to decrease at BCL-140.

3.3.2. Risperidone

Risperidone inhibited HERG current by 11% at the clinical therapeutic unbound concentration of 8 nM (33 ng/ml total; Conley and Mahmoud, 2001) with an IC_{20} value of 11 nM, and had little effect on L-type Ca^{2+} current (Fig. 2, Ca^{2+} $IC_{50}=116000$ nM). In the guinea pig, risperidone caused dose-related decreases in blood pressure and increases in heart rate (Table 2). The increase in basal heart rate did not allow us to use a conditioning pacing cycle of 50 beats at BCL-240 so a BCL-220 cycle was used instead. $MAPD_{50}$ and $MAPD_{90}$ at BCL-200 showed statistically significant reductions with increasing concentrations of drug. Even though very small increase (<2 ms) in alternans

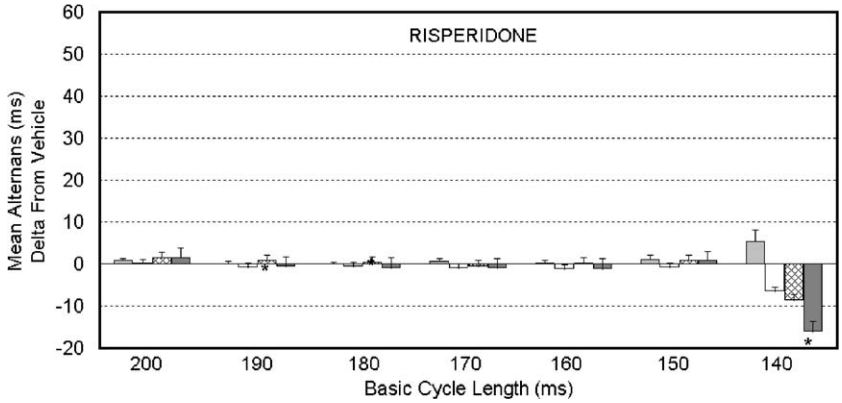

Fig. 8. Mean alternans of beat-to-beat $MAPD_{50}$ in anesthetized guinea pigs ($n=3-6$) treated with risperidone intravenous infusion at free drug plasma concentrations of 6.1 ■, 29.6 □, 131 ☒ and 591 nM). *Denotes significant difference from pre-dose baseline compared to respective vehicle difference from pre-dose baseline at each dose level within a given treatment, $P<0.05$.

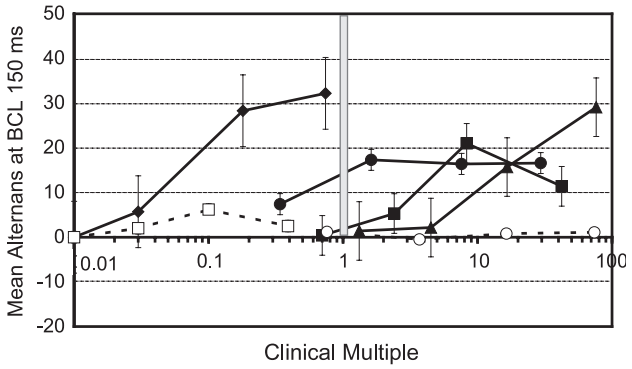

Fig. 9. Mean alternans of beat-to-beat $MAPD_{50}$ in anesthetized guinea pigs (n = 6) during pacing at BCL-150 ms in relation to the effective therapeutic concentration (unbound) in humans (denoted as the line at 1). Proarrhythmic drugs represented by solid line with filled symbols are bepridil (◆), E-4031 (●), cisapride (■) and terfenadine (▲). Drugs rarely associated with Torsade de Pointes represented by dotted line with empty symbols are verapamil (□) and risperidone (○).

was observed at BCL-200, mean alternans was significantly reduced by 16 ms below vehicle control responses at BCL-140 and a concentration of 591 nM (Fig. 8). Risperidone appeared to display antiarrhythmic properties at plasma concentration up to 74-fold the clinical concentrations.

4. Discussion

In this study we quantified changes in action potential alternans on a beat-to-beat basis following administration of selected cardiac and non-cardiac drugs at clinically relevant concentrations in normal anesthetized guinea pigs. This approach was found to differentiate six HERG blockers. E-4031, bepridil, terfenadine and cisapride all increased mean alternans at a cycle length of 150 ms at clinical concentrations associated with higher incidences of fatal tachyarrhythmias (Fig. 9). Verapamil and risperidone showed no increase in mean alternans. The relative rank order of alternans generation in relation to their clinically used concentrations was bepridil > E-4031, terfenadine > cisapride > verapamil > risperidone.

In humans, E-4031 and other class III antiarrhythmics (Fujiki et al., 1994; Lande et al., 1998) produce 10–15% increases in QTc (60–80 ms) at their clinical concentrations. This is very large compared to changes observed with non-cardiovascular agents that have been removed from the marketplace or given black box warnings in their package labeling. Several guinea pigs given E-4031 showed beat-to-beat alternans that reduced quickly upon repeated pulses indicating the potential rapid onset of cardiac memory or accommodation as described by Watanabe and Koller (2002). However, at supratherapeutic concentrations up to 7- to 29-fold or higher, a second oscillatory process of alternans seemed to be occurring during the 30-beat pulsing train (Fig. 4) indicative of discordant alternans (Watanabe et al., 2001). Thus, this second pattern of oscillation may be indicative of cardiac heterogeneity of the epicardial MAPD analogous to wavelet breakup because it always seemed to occur immediately prior to the onset of a Torsade de Pointes pattern at the higher pacing frequencies. Further characterization of this phenomenon is warranted.

Verapamil and bepridil produced very different responses both in vitro and in vivo in relation to their clinical free drug concentrations. Expressing the ratio of inhibition of HERG over that of L-type Ca^{2+} currents by verapamil at the clinical concentration of 34 nM, (HERG IC_{34} nM/Ca IC_{34} nM) yields a value of approximately 1.6, whereas a similarly derived ratio of 120 is obtained with bepridil at its clinical use level of 15 nM. Verapamil is rarely associated with QT prolongation and Torsade de Pointes, whereas normal use levels of bepridil produce approximately 20–30 ms increase in QTc (Singh et al., 1985) and a 0.97% frequency of ventricular arrhythmias associated with its use (Physicians Desk Reference, 2002). These results suggest that inhibition of L-type Ca^{2+} currents at concentrations that also inhibit HERG current (ratio close to 1, as for verapamil) may offset to some extent, the liability associated with inhibition of HERG alone.

Verapamil produced no significant increases in the MAPD or mean alternans at increased pacing cycles with plasma concentrations approximately 56% of its clinical concentration. Higher concentrations produced decreases in resting heart rate and blood pressure (data not reported) that prevented recordings of MAPD. To this latter point, even though these concentrations were less than the clinical levels, it suggests a greater sensitivity in the hemodynamic response in the guinea pig compared to humans, and similar to our previous reported observation in the conscious dog (Fossa et al., 2002). Bepridil, on the other hand, produced concentration-related increases in $MAPD_{50}$ and mean alternans starting below clinical concentrations (0.03–0.73 times) with no significant decreases in blood pressure or resting heart rate. It should be noted that the increased alternans observed with bepridil occurred even at the lowest pacing rate (BCL-200).

Terfenadine and cisapride were both removed from the marketplace due to an increased incidence of Torsade de Pointes (Woosley et al., 1993; Wysowski et al., 2001). For both compounds, their arrhythmogenicity was usually associated with the concurrent administration of CYP-3A4 metabolic inhibitors that caused at least a 3-fold increase in the parent plasma levels of cisapride (Gladziwa et al., 1991) and a 10-fold increase for terfenadine (Honig et al., 1993). There are no known reports of Torsade de Pointes for terfenadine at the therapeutic drug concentration of 0.6 nM, when QTc is prolonged approximately 6 ms (Woosley et al., 1993; Pratt et al., 1996). In the guinea pig, this concentration produced no increase in alternans except at BCL-140 where no changes in mean $MAPD_{200}$ or hemodynamic parameters were observed. At 10-fold greater concentrations, similar to those associated with QTc prolongation and Torsade de

Pointes (Pohjola-Sintonen et al., 1993), we found increases in $MAPD_{90}$ and alternans (17–30 ms). The highest concentration of terfenadine (46 nM) produced decreases in basal heart rate, while alternans increased at all cycle lengths shorter than 200 ms.

Terfenadine was recently studied in an isolated perfused rabbit heart model that not only measures MAPD but also the beat-to-beat MAPD instability (much like alternans) that has purportedly the ability to unfailingly predict proarrhythmia (Hondeghem and Hoffman, 2003). In that study, no effects were observed on either the triangulation of the morphology of the monophasic action potential recordings or instability at 23 (0.5 μg/ml) and 230 (5 μg/ml) times the highest concentration used in our studies. Thus the sensitivity of our guinea pig model appears greater potentially because of other in vivo influencing factors such as hemodynamics, autonomic tone or accounting for steady state protein binding.

Cisapride increased alternans at clinically relevant concentrations. At therapeutic levels of approximately 2 nM, alternans increased only at fast pacing cycles (BCL-140). Threefold increases in plasma concentrations produced increases in MAPD but alternans only increased at shorter pacing cycles of BCL-150, thus suggesting tachycardia-induced arrhythmias. Discordant alternans also frequently occurred with cisapride. As shown with E-4031 in Fig. 4, the discordant alternans at the highest concentrations with cisapride may have affected the quantification of the entire 30-pulse train with periods of oscillations where the net difference between consecutive beats returned to near zero for several pulses resulting in high variance that could account for no statistical significance.

Despite its widespread use for many years, risperidone has an excellent cardiac safety record and only one case of Torsade de Pointes has been reported in the literature (Ravin and Levenson, 1997) even though risperidone can cause QT prolongation at prescribed dosage or at slightly higher levels (PDAC, 2000). The lack of alternans observed, even at the highest concentrations of 591 nM where HERG current was inhibited in HEK293 cells by 80%, indicates that other mechanisms must contribute to mitigate the likelihood of developing arrhythmias. The HERG IC_{50}/Ca IC_{50} ratio for risperidone is >10,000, suggesting that blockade of L-type Ca^{2+} current does not account for the lack of alternans observed. Thus alternative mechanisms, such as intracellular Ca^{2+} handling and/or autonomic tone modulations need to be considered to clarify arrhythmogenic liability of HERG blockers and QT prolonging agents.

Changes in beat-to-beat repolarization have been studied clinically preceding the onset of Torsade de Pointes and related to incidences of fatalities (Leclercq et al., 1988). Locati et al. (1995) found that 98% of Torsade de Pointes episodes were preceded by a typical short–long–short initiating sequence. In each case studied, the pattern showed an escalating cascade develop with two features:(1) a premature beat starting the short–long sequence entraining the ventricular beat, (2) as the amplitude of the short–long–short oscillatory pattern increased, the complexity of the subsequent arrhythmias progressively increased until the onset of Torsade de Pointes. Electrical alternans has also been manifested in the electrocardiogram as microvolt T-wave alternans (Adam et al., 1982) or as beat-to-beat QT heterogeneity (Berger et al., 1997). In both cases, sympathetic activation that increased heart rate also intensified the magnitude of these responses. T-wave alternans is associated with an increased incidence of sudden cardiac death (Bloomfield and Cohen, 2001) and used as a prognostic measure in patients with dilated cardiomyopathy (Kitamura et al., 2002). T-wave alternans is reduced with antiarrhythmics such as amiodarone (Groh et al., 1999) and beta blockers (Klingenheben et al., 2001), but it has not been used for predicting outcomes with non-cardiovascular agents associated with Torsade de Pointes like terfenadine or cisapride.

4.1. Limitations of the study

The results presented in this study describe changes in electrical alternans from hearts of normal healthy animals. Whether or not these results would differ in various diseased states remains to be determined. However, our data suggests that the use of normal animals is justified because prospective evaluation of experimental drugs prior to human exposure dictates that a model is adequately sensitive to predict the concentration-related liability in clinical phase I and II patients that are generally in good health. Furthermore, the vast majority of Torsade de Pointes arrhythmias experienced with non-cardiovascular agents occur in normal healthy patients, usually following drug–drug interactions that raise the plasma concentration of the offending drug or metabolite to undesirable levels. Nonetheless, the guinea pig heart is known to lack the transient outward K^+ current (Inoue and Imanaga, 1993). This current is known to be downregulated in human cardiac disease states such as congestive heart failure (Beuckelmann et al., 1993) and thus may provide additional sensitivity (Hoppe et al., 1999) for manifestation of alternans.

Ca^{2+} handling, heterogeneity in repolarization and conduction velocity were not measured in our studies and are each recognized as important contributing factors to the risk of arrhythmia. Even though alternations in intracellular Ca^{2+} levels have been demonstrated to occur independent of action potential alternans (Walker and Rosenbaum, 2003), the reverse has not. A type of discordant alternans was observed at higher concentrations with all proarrhythmogens in this study, but the relationship between spatial and temporal dispersion and intracellular Ca^{2+} handling and heterogeneity remains to be determined. Finally, Fenton et al. (2003) have shown that when conduction velocity is decreased gradually over a wide range of diastolic intervals, alternans can be decreased or even be suppressed entirely. In addition, while terfenadine has been reported to block sodium current with an IC_{50} of 120 nM (Lu and Wang, 1999), alternans were present in our studies with this drug.

Further, we found that lidocaine, when used at concentrations up to four times the clinically used level, did not affect alternans generated at faster pacing cycles. Therefore further work describing the interaction of sodium and HERG currents on alternans and the role of conduction velocity in this model are necessary.

Finally, plasma electrolyte levels such as K^+ and Mg^{2+} were not measured and could impact the interpretation of the data. However, the drugs that were chosen for this acute study have been extensively studied in other animal models and humans and neither acute nor chronic electrolyte shifts have been reported as the basis for either proarrhythmic or antiarrhythmic activity.

In summary, the study of alternans differentiated four HERG blocking drugs with high degree of arrhythmogenic liability from two of those that are safe in man at pharmacologically relevant concentrations. Although the ionic and cellular mechanisms that underlie the generation of alternans are not fully understood, the study of alternation in cardiac APD may serve as an appropriate approach toward prediction of clinical outcomes.

Acknowledgements

It is with much gratitude that we acknowledge the contributions of Roxanne L. Winslow, and William Gorcyzca for conducting the experimentation using the anesthetized guinea pig, and Walter Volberg, Shuya Wang, David Caouette and Jainlin Feng for in vitro electrophysiological measurements. The contributions of Adam S. Yasgar in generating the plasma concentrations and plasma protein binding and comments from Manfred Hauben are gratefully acknowledged.

References

Adam, D.R., Powell, A.O., Gordon, H., Cohen, R.J., 1982. Ventricular fibrillation and fluctuations in the magnitude of the repolarization vector. I.E.E.E. Comput. Cardiol., 241–244.

Benet, L.Z., 1985. Pharmacokinetics and metabolism of bepridil. Am. J. Cardiol. 55, 8C–13C.

Berger, R.D., Kasper, E.K., Baughman, K.L., Marban, E., Calkins, H., Tomaselli, G.F., 1997. Beat-to-beat QT interval variability: novel evidence for repolarization lability in ischemic and nonischemic dilated cardiomyopathy. Circulation 96, 1557–1565.

Beuckelmann, D.J., Nabauer, M., Erdmann, E., 1993. Alterations of K^+ currents in isolated human ventricular myocytes from patients with terminal heart failure. Circ. Res. 73, 379–385.

Bloomfield, D.M., Cohen, R.J., 2001. Repolarisation alternans. In: Malik, M. (Ed.), Risk of Arrhythmia and Sudden Death. BMJ Book, London, pp. 256–265.

Boudinot, F.D., Jusko, W.J., 1984. Fluid shifts and other factors affecting plasma protein binding of prednisolone by equilibrium dialysis. J. Pharm. Sci. 73, 774–780.

Chen, P.S., Garfinkel, A., Weiss, J.N., Karagueuzian, H.S., 1997. Spirals, chaos, and new mechanisms of wave propagation. Pacing Clin. Electrophysiol. 20, 414–421.

Conley, R.R., Mahmoud, R.A., 2001. Randomized double-blind study of risperidone and olanzapine in the treatment of schizophrenia or schizoaffective disorder. Am. J. Psychiatry 158, 765–774.

De Clerck, F., Van de Water, A., D'Aubioul, J., Lu, H.R., van Rossem, K., Hermans, A., Van Ammel, K., 2002. In vivo measurement of QT prolongation, dispersion and arrhythmogenesis: application to the preclinical cardiovascular safety pharmacology of a new chemical entity. Fundam. Clin. Pharmacol. 16, 125–140.

Fenton, F.H., Cherry, E.M., Hastings, H.M., Evans, S.J., 2003. The APD restitution hypothesis revised: slope >1 does not always determine alternans and spiral wave breakup. P.A.C.E. 389 (abstract).

Franz, M.R., Swerdlow, C.D., Liem, L.B., Schaefer, J., 1988. Cycle length dependence of human action potential duration in vivo. J. Clin. Invest. 82, 972–979.

Fossa, A.A., Depasquale, M.J., Raunig, D.L., Avery, M.J., Leishman, D.J., 2002. The relationship of clinical QT prolongation to outcome in the conscious dog using a beat-to-beat QT-RR interval assessment. J.P.E.T. 202, 828–833.

Fujiki, A., Tani, M., Mizumaki, K., Shimono, M., Inoue, H., 1994. Electrophysiologic effects of intravenous E-4031, a novel class III antiarrhythmic agent, in patients with supraventricular tachyarrhythmias. J. Cardiovasc. Pharmacol. 23, 374–378.

Gilmour Jr., R.F., Chialvo, D.R., 1999. Electrical restitution, critical mass and the riddle of fibrillation. J. Cardiovasc. Electrophysiol. 10, 1087–1089.

Gilmour Jr., R.F., Otani, N.F., Watanabe, M.A., 1997. Memory and complex dynamics in cardiac Purkinje fibers. Am. J. Physiol. 272, H1826–H1832.

Gladziwa, U., Bares, R., Klotz, U., Dakshinamurty, K.V., Ittel, T.H., Seiler, K.-U., Sieberth, H.-G., 1991. Pharmacokinetics and pharmacodynamics of cisapride in patients undergoing hemodialysis. Clin. Pharmacol. Ther. 50, 673–681.

Groh, W.J., Shinn, T.S., Engelstein, E.E., Zipes, D.P., 1999. Amiodarone reduces the prevalence of T wave alternans in a population with ventricular tachyarrhythmias. J. Cardiovasc. Electrophysiol. 10, 1335–1339.

Hondeghem, L.M., Hoffman, P., 2003. Blinded test in isolated female rabbit heart reliably identifies action potential duration prolongation and proarrhythmic drugs: importance of triangulation, reverse use dependence and instability. J. Cardiovasc. Pharmacol. 41, 14–24.

Hondeghem, L.M., Carlsson, L., Duker, G., 2001. Instability and triangulation of the action potential predict serious proarrhythmia, but action potential duration prolongation is antiarrhythmic. Circulation 103, 2004–2013.

Honig, P.K., Wortham, D.C., Zamani, K., Conner, D.P., Mullin, J.C., Cantilena, L.R., 1993. Terfenadine–ketoconazole interaction. Pharmacokinetic and electrocardiographic consequences. J.A.M.A. 269, 1513–1518.

Hoppe, U.C., Johns, D.C., Marban, E., O'Rourke, B., 1999. Manipulation of cellular excitability by cell fusion. Effects of rapid introduction of transient outward K^+ current on the guinea pig action potential. Circ. Res. 84, 964–972.

Inoue, M., Imanaga, I., 1993. Masking of A-type K^+ channel in guinea pig cardiac cells by extracellular Ca^{2+}. Cell Physiol. 33, C1434–C1438.

Kitamura, H., Ohnishi, Y., Okajima, K., Ishida, A., Galeano, E.J., Adachi, K., Yokoyama, M., 2002. Onset heart rate of microvolt-level T-wave alternans provides clinical and prognostic value in nonischemic dilated cardiomyopathy. J. Am. Coll. Cardiol. 39, 295–300.

Klingenheben, T., Gronefeld, G., Li, Y.G., Hohnloser, S.H., 2001. Effect of metoprolol and D,L-sotalol on microvolt-level T-wave alternans. Results of a prospective, double-blind, randomized study. J. Am. Coll. Cardiol. 38, 2013–2019.

Koller, M.L., Riccio, M.L., Gilmour, R.F., 1998. Dynamic restitution of action potential duration during electrical alternans and ventricular fibrillation. Am. J. Physiol. 275, H1635–H1642.

Lande, G., Maison-Blanche, P., Fayn, J., Ghadanfar, M., Coumel, P., Funck-Bretano, C., 1998. Dynamic analysis of dofetilide-induced

changes in ventricular repolarization. Clin. Pharmacol. Ther. 64, 312–321.

Leclercq, J.F., Maison-Blanche, P., Cauchemez, B., Coumel, P., 1988. Respective role of sympathetic tone and cardiac pauses in the genesis of 62 cases of ventricular fibrillation recorded during Holter monitoring. Eur. Heart J. 9, 1276–1283.

Lesko, L.J., Benotti, J.R., Alpert, J.S., Brady, P.M., McCue, J.E., Weiner, B.H., Ockene, I.S., 1986. Pharmacokinetics of intravenous bepridil in patients with coronary disease. J. Pharm. Sci. 75, 952–954.

Locati, E.H., Maison-blanche, P., Dejode, P., Cauchemez, B., Coumel, P., 1995. Spontaneous sequences of onset of Torsade de Pointes in patients with acquired prolonged repolarization: quantitative analysis of Holter recordings. J. Am. Coll. Cardiol. 25, 1564–1575.

Lu, Y., Wang, Z., 1999. Terfenadine block of sodium current in canine atrial myocytes. J. Cardiovasc. Pharmacol. 33, 507–513.

McTavish, D., Goa, K.L., Ferrill, M., 1990. Terfenadine—an updated review of its pharmacological properties and therapeutic efficacy. Drugs 39, 552–574.

Muller, F.B., Ha, H.R., Hotz, H., Schmidlin, O., Follath, F., Buhler, F.R., 1986. Once a day verapamil in essential hypertension. Br. J. Clin. Pharmacol. 21, 143S–147S.

Physicians' Desk Reference ® ©, Vascor (bepridil hydrochloride). The Medical Economics Company, 2002.

Pohjola-Sintonen, S., Viitasalo, M., Toivonen, L., Neuvonen, P., 1993. Itraconazole prevents terfenadine metabolism and increases risk of torsades de pointes ventricular tachycardia. Eur. J. Clin. Pharmacol. 45, 191–193.

Pratt, C.M., Ruberg, S., Morganroth, J., McNutt, B., Woodward, J., Harris, S., Ruskin, J., Moye, L., 1996. Dose–response relation between terfenadine (Seldane) and the QTc interval on the scalar electrocardiogram: distinguishing a drug effect from spontaneous variability. Am. Heart J. 131, 472–480.

Psychopharmacological Drug Advisory Committee (PDAC) 2000. FDA background document on Zeldox (PDAC) 0700.M01). Food and Drug Administration, Rockville, MD, USA.

Ravin, D.S., Levenson, J.W., 1997. Fatal cardiac event following initiation of risperidone therapy. Ann. Pharmacother. 31, 867–870.

Reed-Hagen, A.E., Tsuchiya, M., Shimada, K., Wentland, J.-A., Obach, R.S., 1999. Pharmacokinetics of ezlopitant, a novel non-peptidic neurokinin-1 receptor antagonist in preclinical species and metabolite kinetics of the pharmacologically active metabolites. Biopharm. Drug Dispos. 20 (9), 429–439.

Rosenbaum, D.S., Jackson, L.E., Smith, J.M., Garan, H., Ruskin, J.N., Cohen, R.J., 1994. Electrical alternans and vulnerability to ventricular arrhythmias. N. Engl. J. Med. 330, 235–241.

Schwartz, P.J., Priori, S.G., Spazzolini, C., Moss, A.J., Vincent, G.M., Napolitano, C., Denjoy, I., Guicheney, P., Breithardt, G., Keating, M.T., Towbin, J.A., Beggs, A.H., Brink, P., Wilde, A.A.M., Toivonen, L., Zareba, W., Robinson, J.L., Timothy, K.W., Corfield, V., Wattanasirichaigoon, D., Corbett, C., Haverkamp, W., Schulze-Bahr, E., Lehmann, M.H., Schwartz, K., Coumel, P., Bloise, R., 2001. Genotype–phenotype correlation in the long-QT syndrome gene-specific triggers for life-threatening arrhythmias. Circulation 103, 89–95.

Singh, B.N., Nademanee, K., Feld, G., Piontek, M., Schwab, M., 1985. Comparative electrophysiologic profiles of calcium antagonist with particular reference to bepridil. Am. J. Cardiol. 55, 14C–19C.

van Haarst, A.D., van't Klooster, G.A.E., van Gerven, J.M.A., Schoemaker, R.C, van Oene, J.C., Burggraaf, J., Coene, M.-C., Cohen, A.F., 1998. The influence of cisapride and clarithromycin on QT intervals in healthy volunteers. Clin. Pharmacol. Ther. 64, 542–546.

Volberg, W.A., Koci, B.J., Su, W., Lin, J., Zhou, J., 2002. Blockade of human cardiac K^+ channel human ether-a-go-go related gene (HERG) by macrolide antibiotics. J.P.E.T. 302, 320–327.

Walker, M.L., Rosenbaum, D.S., 2003. Repolarization alternans: implications for the mechanism and prevention of sudden cardiac death. Cardiovasc. Res. 57, 599–614.

Watanabe, M.A., Koller, M.L., 2002. Mathematical analysis of dynamics of cardiac memory and accommodation: theory and experiment. Am. J. Physiol., Heart Circ. Physiol. 282, H1534–H1547.

Watanabe, M.A., Fenton, F.H., Evans, S.J., Hasting, H.M., Karma, A., 2001. Mechanisms for discordant alternans. J. Cardiovasc. Electrophysiol. 12, 196–206.

Webster, R., Allan, G., Anto-Awuakye, K., Harrison, A., Kidd, T., Leishman, D., Phipps, J., Walker, D., 2001. Pharmacokinetic/pharmacodynamic assessment of the effects of E4031, cisapride, terfenadine and terodiline on monophasic action potential duration in dog. Xenobiotica 31, 633–650.

Woosley, R.L., Chen, Y., Freiman, J.P., Gillis, R.A., 1993. Mechanism of the cardiotoxic actions of terfenadine. J.A.M.A. 269, 1532–1536.

Wysowski, D.K., Corken, A., Gallo-Torres, H., Talarico, L., Rodriguez, E.M., 2001. Postmarketing reports of QT prolongation and ventricular arrhythmia in association with cisapride and Food and Drug Administration regulatory actions. Am. J. Gastroenterol. 96, 1698–1703.

Yan, G.-X., Antzelevitch, C., 1998. Cellular basis for the normal T wave and the electrocardiographic manifestations of the long-QT syndrome. Circulation 98, 1928–1936.