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Drug-induced long QT in isolated rabbit Purkinje fibers: importance of action potential duration, triangulation and early afterdepolarizations

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Abstract

In the present study, we investigated three drug-induced long-QT syndromes in isolated rabbit Purkinje fibers in order to identify the relationship of action potential duration (APD), triangulation of action potentials ($APD_{90}-APD_{40}$) and early afterdepolarizations. Isolated rabbit Purkinje fibers were superperfused in Tyrode solution with solvent, indapamide (1×10^{-4} M, an I_{ks} blocker mimicking long QT1), dofetilide (1×10^{-9}, 1×10^{-8} or 1×10^{-7} M, an I_{kr} blocker mimicking long QT2) or anthopleurin (1×10^{-8} M, an inhibitor of the inactivation of the I_{Na+} current mimicking long QT3) ($n=8$ per group) for 25 min, and stimulated at 1 Hz for 20 min and at 0.2 Hz for another 5 min. Indapamide did not change APD and triangulation or elicit early afterdepolarizations even in the presence of β-adrenergic stimulation with isoproterenol. Dofetilide concentration-dependently prolonged APD_{90}, increased triangulation and elicited early afterdepolarizations. Anthopleurin markedly increased APD_{90} as well as triangulation and elicited early afterdepolarizations. The induction of early afterdepolarizations by dofetilide and anthopleurin was associated with a prolongation of APD_{90} or an increase in triangulation, but not with a change in APD_{40}. Moreover, the degree of the increase in the triangulation was larger than that of APD_{90} in long QT2 (dofetilide-induced) and long QT3 (anthopleurin-induced) models in isolated rabbit Purkinje fibers. Our present study indicates that rabbit Purkinje fibers can be used as long QT2 (dofetilide-mimicking) and LQT3 (anthopleurin-mimicking) syndrome models, and confirms that drug-induced long QT1 (indapamide-mimicking) is absent. Our present study also shows the relationship between a prolongation of APD_{90} or increase in triangulation and the induction of early afterdepolarizations with dofetilide (I_{kr} blocker) and anthopleurin (I_{Na} modulator) in isolated rabbit Purkinje fibers.

Keywords: APD (action potential duration); Triangulation; Afterdepolarization, early; Purkinje fiber, rabbit; Long QT syndrome

1. Introduction

Several mutation forms encoding cardiac ion channels were reported to link to the congenital long QT located on certain chromosomes (Chiang and Roden, 2000; Priori et al., 1999; Keating, 1995; Vincent, 1998). Mutations in KCNQ1 and KCNE1 are responsible for defects in the slowly activating component of the delayed rectifier potassium current (I_{ks}), which are involved in long QT1 syndrome. Mutations in HERG (human ether-a-go-go-related gene) are responsible for defects in the rapidly activating component of the delayed rectifier potassium current (I_{kr}), and are involved in long QT2. Mutations in SCN5A result in incomplete inactivation of the sodium channel (I_{Na}) and are responsible for long QT3 (Chiang and Roden, 2000; Priori et al., 1997). This concept has the important implication that therapy may be differential for the different long-QT syndrome, and is important as well to understand the mechanism of the acquired long-QT syndrome (drug-induced long-QT syndrome).

The use of pharmacological models as surrogates for congenital models of long-QT syndrome has been widely propagated, especially in vitro (Antzelevitch, 2001; Rosen, 2001). For example, Antzelevitch's group used pharmacological agents to mimic the long QT1, QT2 and QT3 syndrome in the arterially perfused wedge of canine left ventricle. They used an I_{ks} blocker (chromanol 293B), I_{kr} blocker (D-sotalol or dofetilide) and an inhibitor of the inactivation of the I_{Na} (anthopleurin) as a surrogate for long QT1, QT2 and QT3, respectively, in the studies (Shimizu

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and Antzelevitch, 1997, 2000). Priori et al. (1996) and Studenik et al. (2001) also developed an in vitro model to mimic long QT2 and QT3 by using an I_{kr} blocker (dofetilide) and an inhibitor of the inactivation of the Na^+ current (anthopleurin). In our recent studies, we used dofetilide and quinidine (I_{kr} blockers) to mimic long QT2 in isolated Purkinje fibers (Lu et al., 2000, 2001) and showed that rabbit Purkinje fibers are the most sensitive ones to study drug-induced prolongation of action potential duration (APD) and early afterdepolarizations (EADs) when compared to Purkinje fibers from dog, guinea pigs, pigs, sheep or goat (Lu et al., 2001). However, the drug-mimicking long QT1 and QT2 models in isolated rabbit Purkinje fibers are less known. Furthermore, our preliminary results showed that a I_{ks} blocker could not mimic drug-induced long QT1 in isolated dog Purkinje fibers. Therefore, we used isolated rabbit Purkinje fiber in the present study.

The exact electrophysiological mechanism of TdP arising as a result of long-QT syndrome is still not fully clear. However, both reentries due to dispersion of repolarization and early afterdepolarizations (EADs) have been proposed to be responsible for induction of Torsades de Pointes (TdPs) (Carlsson et al., 1997; Adamantidis et al., 1998; Schechter et al., 1984). The appearance of early afterdepolarizations depends strongly on the action potential duration (APD). Prolongation of APD, either by acquired or congenital long-QT syndrome, can produce the induction of early afterdepolarizations, which are associated with triggered activity and arrhythmias. However, the relationship of the action potential duration (such as APD at 40% and 90% repolarization, APD_{40} and APD_{90} and triangulation of the action potential) and induction of early afterdepolarizations remain elusive in long-QT syndrome. The triangulation of the APD was shown to be an additional potential indicator for the induction of abnormal beats in isolated rabbit hearts (Hondeghem et al., 2001). It can, therefore, be used for detecting drug-induced cardiac arrhythmias in isolated rabbit hearts (Hondeghem et al., 2001).

In addition to in vivo measurements, cardiac electrophysiological tests both in isolated cells or cell lines transected with a specific channel and isolated cardiac tissues are recommended for detecting the potential for QT prolongation and proarrhythmias by a noncardiovascular new chemical entity (NCE) (De Clerck et al., 2002; Haverkamp et al., 2000; Anonymous, 1997). Each individual test has some advantages and disadvantages. At least four functional cell types in the ventricles (epicardial, midmyocardial-M cell, endocardial-like papillary muscles and Purkinje fibers) can be used to detect drug-induced long APD and early afterdepolarizations. M cells, similar to Purkinje fibers, are most sensitive to detect drug-induced long APD and early afterdepolarizations (Shimizu and Antzelevitch, 1997, 2000; Antzelevitch, 2001). Due to the M-cells in rabbit hearts being unknown and for technical reasons, we investigated three pharmacological models of long-QT syndrome only in isolated rabbit Purkinje fibers in the present study in order to (1) identify the relationship between APD, triangulation and early afterdepolarizations, (2) enlarge previous studies in isolated Purkinje fibers (Lu et al., 2000, 2001) and (3) describe a test in vitro for detecting potential drug-induced QT prolongation and proarrhythmias. We used indapamide (I_{ks} blocker) for long QT1, dofetilide (I_{kr} or HERG channel blocker) as a surrogate for the long QT2 and anthopleurin (an augmentation of late I_{Na}) for mimicking long QT3.

2. Materials and methods

2.1. Electrophysiological measurements

Electrophysiological experiments were performed on isolated rabbit Purkinje fibers with conventional microelectrode techniques and under the guidelines of the European Convention on the Protection of Vertebrate Animals. Purkinje fibers were isolated from the ventricles of female New Zealand White rabbits (weighing 1.8–2.5 kg). The approach we used is similar to that in our recent studies (Lu et al., 2000, 2001) and to that of others (Carlsson et al.,1997; Adamantidis et al., 1995). Briefly, cardiac tissue was isolated within 5 min after sacrifice of the animal and stored in a tissue bath. The Purkinje fibers were perfused with a gassed (95% O_2 and 5% CO_2). Tyrode solution having the following composition (in mM): NaCl 136.9, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1.04, $NaHCO_3$ 11.9, NaH_2PO_4 0.42 and glucose 5.5; pH 7.4 ± 0.15.

The preparations were fixed at the bottom of a 2.5-ml perfusion organ bath by thin cotton threads and continuously superfused with oxygenated Tyrode solution at a rate of 1.8 ml/min and with solutions of the study compound or solvent at a rate of 200 μl/min. The temperature in the bath was kept at 36.5 ± 1.0 °C.

The Purkinje fibers were stimulated at a basal rate of 1 Hz (60 beats/min or 1000 ms cycle length) through bipolar Teflon-coated silver wire electrodes that were connected to a pulse generator and an isolation transformer. Stimuli consisted of regular pulses of a duration of 1–2 ms delivered at an intensity of two times the diastolic threshold. Intracellular potentials were recorded with glass microelectrodes filled with 2.7 M KCl, with tip resistances between 5 and 35 MΩ. The microelectrode was connected to the headstage of the microelectrode amplifier (Hugo Sachs Elektronik: HSE; type 695; March-Hugstetten, Germany). Action potentials were acquired by a action potential software (Janssen Pharmaceutical, Beerse, Belgium) at a sampling rate of 20 kHz, filtered at 100 Hz. They were stored on a personal computer for later analysis. The electrode was placed on different places of the preparation till a normal and stable action potential was induced. Thereafter, the electrode was usually continuously placed in the same place of the preparation. Electronic differentiation was used to obtain the maximum rate of rise of the action potential (V_{max} in V/s). The resting membrane potential (in mV), amplitude of

action potential (in mV) and action potential duration (APD) at 40%, 50% or at 90% repolarization (APD_{40}, APD_{50} or APD_{90} in ms) were determined from the recordings. To determine the effective refractory period (in ms) and recovery time of the action potential (in ms), premature action potentials were elicited through the delivery of extra stimuli (S_2). Effective refractory period (ERP) was measured by means of S_2 after every three stimuli, and the time between the pacing stimulus and extra stimulus, was increased until a propagated response was produced. Recovery time of the action potential (RT) was determined when the AAP of a premature action potential reached above 95% of the amplitude of action potential of the previous complete action potential. Triangulation of the action potential is defined as the difference between the duration of action potential at 90% repolarization and that of 40% of repolarization ($APD_{90}-APD_{40}$, in ms). This parameter presents an accurate indicator for changes in action potential morphology during phases two to three of repolarization. Triangulation of the action potentials is a key phenomenon in the development of early afterdepolarization (Hondeghem et al., 2001).

Control values (baseline values before contact with solvent or compound) were determined after a stabilization period (about 60–90 min), when the measured variables had reached a steady state. The electrophysiological parameters were again measured at 20 min (at 1 Hz) and 25 min (at 0.2 Hz) after continuous infusion of the compound or solvent. When the preparation exhibited early afterdepolarizations, APD_{90} was usually prolonged to more than 1000 ms, and it was taken as 1000 ms so that its mean values could be calculated (Adamantidis et al., 1995). Effective refractory period and recover time could not be measured in case of the Purkinje fibers with early afterdepolarizations.

Abnormal action potentials in terms of triggered activities were also recorded during the experiments. Triggered activities are caused by early afterdepolarizations. We defined early afterdepolarizations on the basis of features recommended by our recent studies (Lu et al., 2000, 2001) and by others (Damiano and Rosen, 1984; January et al., 1991): an early afterdepolarization was identified as a small afterpotential that interrupts or delays the normal repolarization of the action potential.

2.2. Experimental protocols

After baseline values were recorded, solvent, indapamide at 1×10^{-3} M (an I_{ks} blocker for mimicking long QT1) (Turgeon et al., 1994), dofetilide (1×10^{-8}, 1×10^{-7} or 1×10^{-6} M; an I_{kr} blocker for mimicking long QT2) (Abrahamsson et al., 1996) or anthopleurin (1×10^{-7} M; an augmentation of the late I_{Na} for mimicking long QT3) (Shimizu and Antzelevitch, 1997, 2000; Studenik et al., 2001) was continuously superfused into the Tyrode solution in the chamber near the tissue bath for 25 min in Purkinje fibers ($n=8$ per group). Infusion of solvent or of the compound at a rate of 200 μl/min into the Tyrode solution at a rate of 1800 μl/min yielded a dilution of 1/10. Therefore, a final concentration of indapamide was 1×10^{-4} M, of anthopleurin was 1×10^{-8} M and concentrations of dofetilide were 1×10^{-9}, 1×10^{-8} and 1×10^{-7} M, in the tissue bath, respectively. Isoproterenol (at a final concentration of 2×10^{-7} M; $n=6$) alone and in the presence of indapamide (at a final concentration of 1×10^{-4} M; $n=6$) were also investigated in the same protocol. The compound concentrations, used in the present study, were previously shown to block their respected ion currents in myocardial cells or from other studies in vitro (Shimizu and Antzelevitch, 1997, 2000; Studenik et al., 2001; Lu et al., 2000; Turgeon et al., 1994). The electrophysiological parameters were measured at 20 min after the start of the infusion in isolated rabbit Purkinje fibers stimulated at 1 Hz. Subsequently, the stimulation rate was reduced to a low frequency (0.2 Hz = 12 beats/min) for another 5 min in order to increase the detection sensitivity for drug-induced abnormal action potentials such as EADs in a condition of extreme bradycardia (Carlsson et al., 1997; Adamantidis et al., 1995). The experiments were made to randomize systematically between groups in the study in order to avoid to use the Purkinje fibers from the same rabbit in the one group.

2.3. Drugs

Indapamide and anthopleurin were purchased from Sigma (St. Louis, USA) and dofetilide was obtained from Pfizer Central Research (Kent, England). To retard oxidation of isoproterenol (Sigma), ascorbic acid was added in at a proportion of 1–20 (isoproterenol to ascorbic acid). All compounds were dissolved in pyrogen-free water and 9% mannitol, acidified with tartaric acid to obtain a pH of 4. The same solution without compound was used as the solvent control.

2.4. Data analysis

Because all data are not clearly distributed in a Gaussian manner, small samples and some values (for example, APD_{90}) 'off the scale' in case of the early afterdepolarizations, we applied a nonparametric test for the present study. All values are expressed as median (minimum and maximum). In order to evaluate drug-induced effects relative to the normal variation, differences in the Δ changes of the baseline values between the solvent-control group and drug-treated group were evaluated for statistical significance by means of the Wilcoxon–Mann–Whitney *U*-test. For the evaluation of the difference between the incidence of early afterdepolarizations in the different groups, Fisher's exact test was used. Two-tailed probabilities of less than 0.05 were considered to reflect a statistically significant difference. The statistically significant difference in the Δ changes of the baseline values between the solvent group and drug group are presented in tables with the absolute values.

3. Results

3.1. Effects of indapamide (an I_{ks} blocker for mimicking long QT1) on action potentials

3.1.1. Effects of indapamide alone on the action potential

Twenty-five-minute continuous infusion of indapamide (1×10^{-4} M; $n=8$) in the absence of isoproterenol did not significantly alter any action potential parameters measured, including the amplitude of the action potential (AAP), the action potential duration at 40%, 50% or 90% repolarization (APD_{40}, APD_{50} or APD_{90}), the resting membrane potential of the action potential, the effective refractory period, the recovery time of the action potential and the maximum rate of depolarization during the upstroke of the action potential (V_{max}) relative to the solvent control group ($n=8$), both in a condition of a normal rhythm (a stimulation rate of 1 Hz = 60 beats/min) for 20 min and of bradycardia (a stimulation rate of 0.2 Hz = 12 beats/min) for another 5 min (Table 1).

A 25-min continuous infusion of indapamide (1×10^{-4} M; $n=8$) did not elicit early afterdepolarizations (EADs) in any of the eight preparations, not only in a condition of a normal rhythm (1 Hz) but also in a condition of bradycardia (0.2 Hz) (versus zero out of eight preparations with solvent; $P>0.05$) (Table 3).

3.1.2. Effects of indapamide in the presence of isoproterenol or effects of isoproterenol alone on the action potential

A 20-min continuous infusion of indapamide (1×10^{-4} M) in the presence of isoproterenol (2×10^{-7} M) ($n=6$) significantly reduced the triangulation, APD_{50}, APD_{90}, effective refractory period and recovery time by 25%, 43%, 34%, 31% and 31% of baseline (versus −2%, −5%, −4%, −3% and −5% of baseline with solvent; $P<0.05$) at a stimulation of 1 Hz. At the end of 25-min infusion and in a condition of extreme bradycardia, indapamide (1×10^{-4} M) in the presence of isoproterenol (2×10^{-7} M) still yielded lower values of the triangulation, APD_{50}, APD_{90}, effective refractory period and of recover time (+9%, −21%, −13%, −15% and −17% of baseline versus +39%, +23%, +32%, +27% and +30% with solvent only; $P<0.05$).

A 20-min continuous infusion of isoproterenol alone (2×10^{-7} M; $n=6$) also reduced the triangulation, APD_{50}, APD_{90}, effective refractory period and recovery time by 18%, 25%, 35%, 34% and 33% of baseline ($P>0.05$ versus those in the group with indapamide in the presence of isoproterenol; $P<0.05$ versus solvent only) at a stimulation rate of 1 Hz. At the end of 25-min infusion and in a condition of extreme bradycardia, isoproterenol alone had similar effects on the triangulation, APD_{50}, APD_{90}, effective refractory period and on the recovery time to those with the group, treated with indampamide in the presence of isoproterenol (−5%, −11%, −27%, −25% and −23% of baseline versus −18%, −25%, −35%, −34% and −33%; $P>0.05$). Infusion of isoproterenol alone or of in the presence of indapamide did not significantly change the amplitude of the action potential, resting membrane potential and V_{max} (data not shown).

Infusion of isoproterenol (2×10^{-7} M) or of indapamide (1×10^{-4} M) in the presence of isoproterenol (2×10^{-7} M) elicited early afterdepolarizations in one out of six preparations (Table 3).

3.2. Effects of dofetilide (an I_{kr} blocker for mimicking long QT2) on action potentials

A 20-min continuous infusion of dofetilide (1×10^{-9}, 1×10^{-8} or 1×10^{-7} M; $n=8$ per concentration) did not significantly alter the amplitude of the action potential, resting membrane potential or V_{max} in isolated rabbit Purkinje fibers stimulated at 1 Hz (60 beats/min) when compared to solvent (Tables 1 and 2) (data from dofetilide at 1×10^{-8} and 1×10^{-9} M are not shown in the table). However, the compound concentration-dependently

Table 1
Effects of solvent and of indapamide (1×10^{-4} M) (an I_{ks} blocker for mimicking long QT1) on isolated rabbit Purkinje fibers incubated with Tyrode solution containing 4 mM KCl and stimulated at 1 Hz (60 pulses/min) and 0.2 Hz (12 pulses/min)

Parameters	Solvent ($n=8$)			Indapamide (1×10^{-4} M) ($n=8$)		
	Baseline/1 Hz	20 min/1 Hz	25 min/0.2 Hz	Baseline/1 Hz	20 min/1 Hz	25 min/0.2 Hz
AAP (mV)	126 (116–137)	125 (119–130)	125 (112–132)	127 (111–132)	124 (109–133)	123 (116–131)
APD_{40} (ms)	130 (88–205)	145 (85–200)	184 (85–250)	154 (100–190)	113 (80–170)	150 (100–205)
APD_{50} (ms)	196 (146–276)	184 (134–262)	244 (147–349)	199 (159–246)	178 (127–211)	233 (136–256)
APD_{90} (ms)	253 (192–346)	242 (171–347)	318 (209–508)	267 (212–313)	235 (175–275)	315 (203–350)
RMP (−mV)	90 (84–91)	91 (79–96)	86 (71–94)	88 (86–93)	91 (85–96)	89 (81–95)
ERP (ms)	245 (197–346)	241 (186–346)	318 (209–508)	255 (217–309)	244 (196–289)	307 (188–329)
RT (ms)	261 (213–362)	249 (186–346)	344 (233–524)	280 (217–325)	255 (196–313)	326 (217–369)
V_{max} (v/s)	631 (526–784)	642 (549–754)	648 (512–766)	616 (173–750)	665 (332–717)	663 (246–746)

Values are median (minimum and maximum). AAP: the amplitude of the action potential. APD_{40}, APD_{50} and APD_{90}: the duration of the action potential at 40%, 50% and 90% repolarizations, respectively. RMP: resting membrane potential. ERP: effective refractory period. RT: recovery time of the action potential. In case of preparations with early afterdepolarizations, APD_{90} was taken as 1000 ms, while APD_{50} was taken as 500 ms in order to calculate their mean values. V_{max}: the maximum rate of depolarization during the upstroke.

Table 2
Effects of dofetilide (1×10^{-7} M) (an I_{kr} blocker for mimicking long QT2) and anthopleurin (1×10^{-8} M) (augmentation of the late I_{Na} for long QT3) on isolated rabbit Purkinje fibers incubated with Tyrode solution containing 4 mM KCl and stimulated at 1 Hz (60 pulses/min) and 0.2 Hz (12 pulses/min)

Parameters	Dofetilide (1×10^{-7} M; $n=8$)			Anthopleurin (1×10^{-8} M; $n=8$)		
	Baseline/1 Hz	20 min/1 Hz	25 min/0.2 Hz	Baseline/1 Hz	20 min/1 Hz	25 min/0.2 Hz
AAP (mV)	133 (113–139)	127 (122–140)	132 (123–138)	126 (119–135)	123 (105–157)	124 (101–150)
APD_{40} (ms)	107 (50–230)	150 (30–225)	0 (0–350)	195 (70–260)	195 (0–475)	263 (0–500)
APD_{50} (ms)	201 (163–218)	284 (179–541)*	422 (395–423)*	257 (146–372)	268 (132–689)*	500 (180–580)*
APD_{90} (ms)	266 (203–315)	472 (307–758)*	597 (431–1000)*	334 (215–444)	389 (242–769)*	805 (561–1000)*
RMP ($-$mV)	86 (84–90)	89 (94–80)	88 (79–90)	89 (84–96)	88 (82–92)	89 (82–92)
ERP (ms)	257 (204–308)	436 (268–668)*	[a]	329 (224–425)	403 (236–675)*	699 (528–769)*
RT (ms)	360 (228–522)	466 (284–700)*	[a]	337 (24–441)	403 (236–675)*	688 (528–840)*
V_{max} (v/s)	508 (326–651)	396 (325–705)	399 (323–721)	615 (417–754)	581 (231–1190)	589 (429–984)

Values are median (minimum and maximum). AAP: the amplitude of the action potential. APD_{40}, APD_{50} and APD_{50}: the duration of the action potential at 40%, 50% and 90% repolarization, respectively. RMP: resting membrane potential. ERP: effective refractory period. RT: recovery time of the action potential. V_{max}: the maximum rate of depolarization during the upstroke. In case of preparations with early afterdepolarizations, APD_{90} was taken as 1000 ms, while APD_{50} was taken as 500 ms in order to calculate their mean values.

[a] The parameters could not be measured because most of the preparations developed early afterdepolarizations.

* $P<0.05$ versus solvent in the Table 1.

prolonged the APD_{50} and APD_{90}, effective refractory period and recovery time. APD_{50} was significantly prolonged by 16%, 67% and 192% of baseline (versus -7% with solvent), and APD_{90} by 19%, 83% and 186% (versus -5% with solvent) by the compound at 1×10^{-9}, 1×10^{-8} or 1×10^{-7} M, respectively, in a condition of a normal rhythm (at a stimulation rate of 1 Hz). The effective refractory period and recovery time were also increased by 17–169% of baseline by dofetilide at 1×10^{-9}, 1×10^{-8} or 1×10^{-7} M (versus -3% and -5% with solvent) in isolated rabbit Purkinje fibers stimulated at 1 Hz. At 1×10^{-9}, 1×10^{-8} and 1×10^{-7} M, the compound increased APD_{40} by 14%, 32% and 32%, respectively, of baseline (versus $+3\%$ with solvent) during a normal rhythm (1 Hz). Furthermore, triangulation was markedly prolonged by 26%, 217% and 400% of the baseline value by the compound at 1×10^{-9}, 1×10^{-8} and 1×10^{-7} M (versus -2% with solvent; $P<0.05$), respectively.

At the end of a 25-min continuous infusion under extreme bradycardia (0.2 Hz = 12 beats/min), the reverse rate dependent effects of dofetilide on APD_{50} and APD_{90} were clearly observed. Dofetilide substantially increased APD_{50} by 56%, 109% and 142% of baseline at 1×10^{-9}, 1×10^{-8} or 1×10^{-7} M (versus 23% of baseline with solvent; $P<0.05$), respectively. The compound prolonged APD_{90} by 75%, 174% and 299% of baseline (versus 32% of baseline with solvent; $P<0.05$) at 1×10^{-9}, 1×10^{-8} or 1×10^{-7} M, respectively. At 1×10^{-9}, 1×10^{-8} and 1×10^{-7} M, the compound changed APD_{40} by $+38\%$, $+99\%$ and -100% of baseline (versus $+32\%$ of baseline with solvent) during bradycardia (0.2 Hz). Furthermore, triangulation was markedly increased by 128%, 279% and 584% of baseline by the compound at 1×10^{-9}, 1×10^{-8} and 1×10^{-7} M, respectively (versus 39% of baseline with solvent; $P<0.05$). The effective refractory period and recovery time were also markedly increased by about 100% of the baseline value by the compound at 1×10^{-9} or 1×10^{-8} M at a stimulation rate of 0.2 Hz (versus $\pm 30\%$ of baseline with solvent; $P<0.05$). The effective refractory period and recover time of the action potential could not be measured because most Purkinje fibers developed early afterdepolarizations with dofetilide at 1×10^{-7} M in this bradycardic condition (0.2 Hz).

A 20-min continuous infusion of dofetilide at 1×10^{-9} M ($n=8$) at a stimulation rate of 1 Hz did not elicit early afterdepolarizations in any of the eight preparations, and the compound at 1×10^{-8} M induced early afterdepolarizations in one out of eight preparations (versus zero out of eight preparations with the solvent; $P>0.05$). In similar conditions, dofetilide at 1×10^{-7} M induced early afterdepolarizations in three out eight preparations in rabbit Purkinje fibers stimulated at 1 Hz ($P<0.05$ versus solvent).

Under extreme bradycardia at a stimulation rate of 0.2 Hz, dofetilide concentration-dependently elicited early afterdepolarizations in one out of eight (13%), in four out of eight (50%) and in seven out of eight preparations (88%) at 1×10^{-9}, 1×10^{-8} and 1×10^{-7} M, respectively (versus 0% with solvent; $P<0.05$ at 1×10^{-8} and 1×10^{-7} M). Fig. 1A shows an example of the effects of solvent on the action potential tracings in a rabbit Purkinje fiber. Fig. 1B shows an example of the effects of dofetilide (1×10^{-7} M) on the action potential tracings in a rabbit Purkinje fiber, with a marked prolongation of APD and early afterdepolarizations in the condition of bradycardia (0.2 Hz). Early afterdepolarizations, induced by dofetilide at 1×10^{-7} M in seven out of eight preparations, were initiated from a take-off potential (the most negative plateau voltage reached before the depolarization of the early afterdepolarization) of -41 mV (from -42 to -31 mV) and reached a peak voltage of -29 mV (from -38 to -14 mV). The early afterdepolarizations, induced by the compound at 1×10^{-8} M, were initiated from a

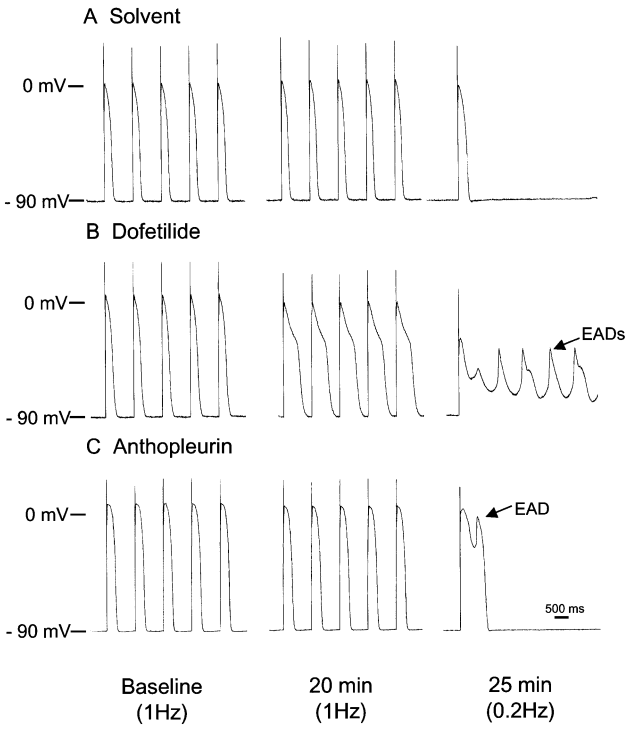

Fig. 1. Representative examples of the effects of solvent (A), dofetilide (1×10^{-7} M mimicking long QT2) (B) and anthopleurin (1×10^{-8} M mimicking long QT3) (C) on the action potential in isolated rabbit Purkinje fibers. Left: baseline at a stimulation rate of 1 Hz and showing normal action potentials. Middle: at 20-min exposure to solvent or compound at a stimulation rate of 1 Hz. Right: at 25-min exposure to solvent or to compound at a stimulation rate of 0.2 Hz and showing early afterdepolarizations (EADs) with dofetilide (1×10^{-7} M) and anthopleurin (1×10^{-8} M).

take-off potential at −33 mV (from −37 to −23 mV). The early afterdepolarizations, induced by dofetilide at 1×10^{-7} M, were more than five successive early afterdepolarizations (sustained triggered activities) in most cases (Table 3).

3.3. Effects of anthopleurin (an augmentation of late I_{Na} for mimicking long QT3) on action potentials

A 20-min continuous infusion of anthopleurin (1×10^{-8} M; $n=8$) did not significantly alter the amplitude of the action potential, resting membrane potential, APD_{40} or V_{max} in isolated rabbit Purkinje fibers stimulated at 1 Hz (60 beats/min) when compared to solvent (Tables 1 and 2). However, the compound moderately prolonged the APD_{50}, APD_{90}, effective refractory period and recovery time by 26%, 36%, 36% and 33%, respectively, of baseline (versus −5%, −4%, −3% and −5% with solvent; $P<0.05$) (Tables 1 and 2). Furthermore, triangulation was markedly increased by 86% of baseline by the compound (versus −2% with solvent; $P<0.05$). This increase in the triangulation is about three times larger than that in APD_{50} or APD_{90} by the compound (versus about one time with solvent).

At the end of a 25-min continuous infusion, under extreme bradycardia (0.2 Hz = 12 beats/min), the reverse rate dependent effects of anthopleurin (1×10^{-8} M) on APD_{50}, APD_{90}, effective refractory period and recover time were marked. Anthopleurin substantially increased the APD_{50}, APD_{90}, effective refractory period, recovery time and the triangulation by 73%, 155%, 142%, 133% and 249% of baseline, respectively (versus 23%, 32%, 27%, 30% and 39% with solvent; $P<0.05$), in a condition of bradycardia (0.2 Hz). AAP, APD_{40}, resting membrane potential and V_{max} were not significantly changed by the compound under extreme bradycardia when compared to solvent (Tables 1 and 2).

A 20-min continuous infusion of anthopleurin (1×10^{-8} M) did not elicit early afterdepolarizations in the eight preparations tested (versus zero out of eight preparations in the solvent). However, under extreme bradycardia at a stimulation rate of 0.2 Hz, anthopleurin at 1×10^{-8} M (contact for 25 min) elicited early afterdepolarizations in six out of eight (75%) preparations (versus 0% with solvent;

Table 3
Incidence of early afterdepolarizations (EADs) induced by different pharmacological interventions on isolated rabbit Purkinje fibers incubated with Tyrode solution containing 4 mM KCl and stimulated at 1 Hz (60 pulses/min) for 20 min and 0.2 Hz (12 pulses/min) for another 5 min (total time 25 min)

Treatments	Incidence of EADs [*n*/*n* (%)]	Onset time of EADs (min)	EAD take-off potential (mV)	Peak voltage of EADs (mV)	Incidence of STA [*n*/*n* (%)]
Solvent ($n=8$)	0/8 (0%)	/	/	/	0/8 (0%)
Indapamide (1×10^{-4} M; $n=8$)	0/8 (0%)	/	/	/	0/8 (0%)
Isoproterenol (2×10^{-7} M; $n=6$)	1/6 (17%)	12	−22	−19	0/6 (0%)
Isoproterenol (2×10^{-7} M) + Indapamide (1×10^{-4} M) ($n=6$)	1/6 (17%)	22	−21	−18	0/6 (0%)
Dofetilide: 1×10^{-9} M ($n=8$)	1/8 (13%)	23	−25	−26	0/8 (0%)
1×10^{-8} M ($n=8$)	4/8 (50%)*	22 (19/25)	−33 (−37/−23)	−10 (−23/+16)	2/4 (50%)
1×10^{-7} M ($n=8$)	7/8 (88%)*	21 (8/25)	−41 (−85/−31)	−29 (−38/−14)	6/7 (86%)*
Anthopleurin 1×10^{-8} M ($n=8$)	6/8 (75%)*	25 (21/25)	−36 (−46/−18)	−17 (−39/+6)	1/6 (17%)

The values of the onset time of EADs, EADs take − off potential and peak voltage of EADs are median (minimal/maximal). STA: sustained triggered activities (more than five successive EADs).

* $P<0.05$ versus solvent.

$P<0.05$) (Table 3). Fig. 1C shows an example of the effects of anthopleurin (1×10^{-8} M) on the action potential tracings in a rabbit Purkinje fiber, with a marked prolongation of APD and early afterdepolarization in a condition of bradycardia (0.2 Hz). Early afterdepolarizations, induced by the compound at 1×10^{-8} M in six out of eight preparations, were initiated from a take-off potential at −36 mV (from −46 to −18 mV) and reached a peak voltage of −17 mV (from +6 to −39 mV). Early afterdepolarizations, induced by anthopleurin at 1×10^{-8} M, consisted of less than three successive early afterdepolarizations in most cases (Table 3).

3.4. Relationship between the incidence of early afterdepolarizations and the action potential duration

Fig. 2 shows the relationship between the incidence of early afterdepolarizations and the action potential duration.

Dofetilide (for long QT2) concentration-dependently prolonged APD_{90} by 73%, 184% and 273%, and increased triangulation by 128%, 279% and 587% at 1×10^{-9}, 1×10^{-8} and 1×10^{-7} M, respectively. These increases in APD_{90} and triangulation were associated with a concentration-dependent augmentation of early afterdepolarizations induced by the compound at 0.2 Hz (13% at 1×10^{-9} M, 50% at 1×10^{-8} M and 88% at 1×10^{-7} M). However, the change of APD_{40}, induced by dofetilide, was not associated with a high incidence of early afterdepolarizations (Fig. 2 and Table 2). At 1×10^{-7} M, dofetilide in fact reduced APD_{40} by 100% versus baseline and yet elicited a 88% incidence of early afterdepolarizations during bradycardia (0.2 Hz).

Anthopleurin at 1×10^{-8} M (for long QT3) prolonged APD_{90} by 138% and increased triangulation by 249%, and elicited 75% incidence of early afterdepolarizations at a stimulation rate of 0.2 Hz. Noticeably, the increase in triangulation was much larger than that of APD_{90}. By contrast, APD_{40} was decreased by 100% versus baseline with dofetilide at 1×10^{-7} M and increased by 42% with anthopleurin (1×10^{-8} M) (Fig. 2).

Indapamide at 1×10^{-4} M (for long QT1) did not significantly alter APD_{40}, APD_{50}, APD_{90} or triangulation when compared to solvent, nor did it induce early afterdepolarizations (Fig. 2), even in the presence of isoproterenol (2×10^{-7} M) (not in Fig. 2).

Furthermore, the increase in triangulation, induced by dofetilide and anthopleurin, is correlated with a prolongation of the APD_{90}, but not with changes of APD_{40} in a condition of a normal rhythm (at 20 min during infusion at a stimulation rate of 1 Hz) and of bradycardia (at the end of 25-min infusion at a stimulation rate of 0.2 Hz) (Fig. 3). Regression analysis showed a relationship between the prolongation of the APD_{90} and those of the triangulation in the pharmacological models for long QT2 (dofetilide) and long QT3 (anthopleurin) in isolated rabbit Purkinje fibers ($R^2=0.63$, $P<0.05$ at 20 min during infusion at a stimulation rate of 1 Hz; $R^2=0.53$, $P<0.05$ at the end of 25-min infusion at a stimulation rate of 0.2 Hz). However, regression analysis showed that there was no relationship between

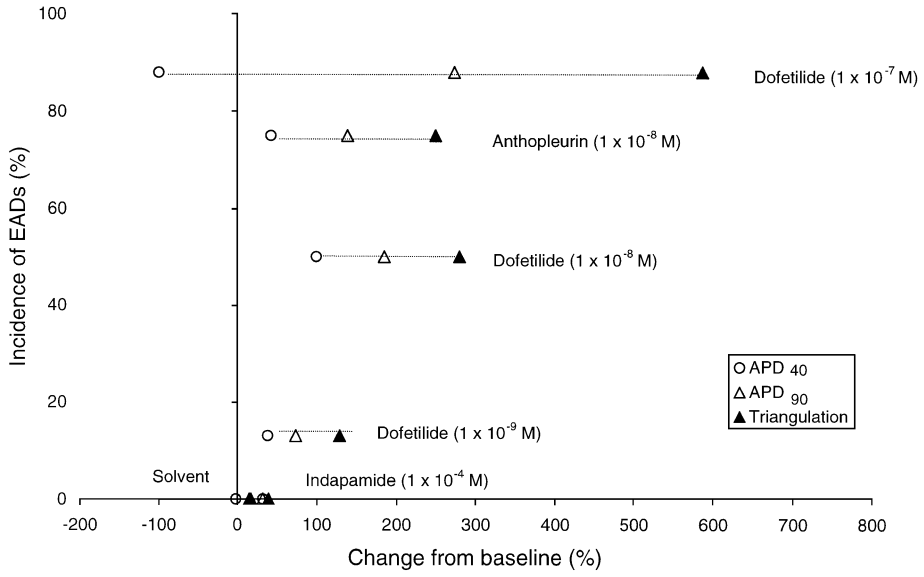

Fig. 2. Effects of a 25-min continuous infusion of solvent, indapamide (1×10^{-4} M mimicking long QT1), dofetilide (1×10^{-9}, 1×10^{-8} and 1×10^{-7} M mimicking long QT2) and anthopleurin (1×10^{-8} M mimicking long QT3) on the duration of the action potential at 40% and 90% repolarization (APD_{40} and APD_{90}), triangulation ($APD_{90}-APD_{40}$) and the incidence of early afterdepolarizations (EADs) in isolated rabbit Purkinje fibers. Values of APD_{40}, APD_{90} and triangulation are expressed as median values and as a percentage change of baseline, and the value of incidence of early afterdepolarizations is expressed as percentage ($n/n\times100\%$). Prolongation of the APD_{90} or increase in triangulation is associated with the incidence rate of early afterdepolarizations.

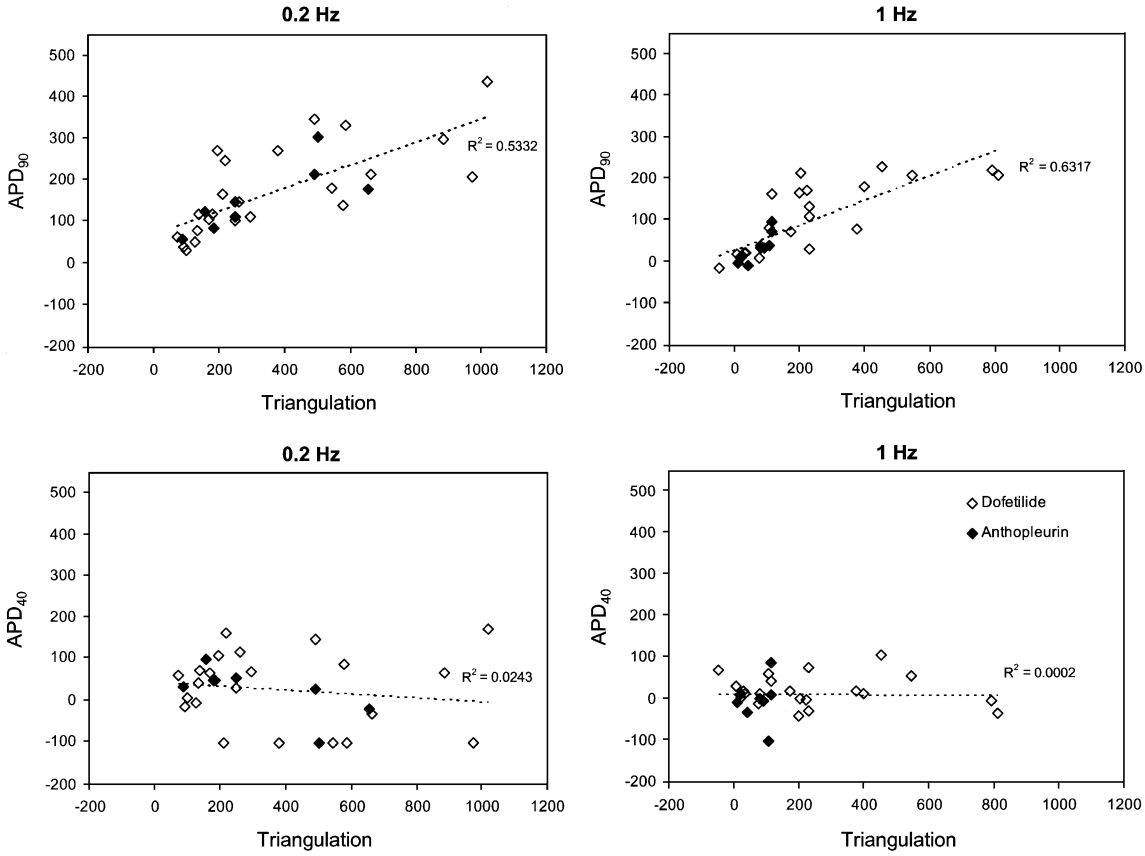

Fig. 3. Simple linear regression analysis between the changes in triangulation relative to the changes in the duration of the action potential at 90% of repolarization (APD_{90}) or at 40% repolarization (APD_{40}) in the pharmacological models for long QT2 (dofetilide) and long QT3 (anthopleurin) at 20 min during infusion at a stimulation rate of 1 Hz and at the end of 25-min infusion at a stimulation rate of 0.2 Hz in isolated rabbit Purkinje fibers. Results are expressed as median values and as a percentage change of baseline. (■) Indapamide, (□) dofetilide.

the changes of the APD_{40} and those of the triangulation ($R^2 = 0.0002$, $P > 0.05$ at 20 min during infusion at stimulation rate of 1 Hz; $R^2 = 0.03$, $P > 0.05$ at the end of 25-min infusion at a stimulation rate of 0.2 Hz) (Fig. 3).

4. Discussion

Our present study shows that appropriate measurement of action potential characteristics in isolated rabbit Purkinje fibers readily detects abnormal repolarizations of action potential in terms of a prolongation of APD_{90}, increase in triangulation ($APD_{90} - APD_{40}$) and of incidence of early afterdepolarizations, induced by dofetilide (I_{kr} blocker mimicking long QT2) and anthopleurin (I_{Na} enhancement mimicking long QT3), but not by indapamide (I_{ks} blocker mimicking long QT1). Moreover, it corroborates the results in isolated rabbit hearts (Hondeghem et al., 2001) showing that drug-induced changes in the action potential slope and morphology during phases two to three of repolarization (triangulation and APD_{90}) rather than the change in APD_{40} are sensitive parameters to detect the proarrhythmic potentials, induced by dofetilide and anthopleurin.

Indeed, controversial results with I_{ks} blockers on the ventricular repolarization (APD/QT interval) in different species have been published. Antzelevitch's group showed that the I_{ks} blocker chromanol 293B mimics long QT1 in isolated dog ventricular cells, but does not mimic long QT1 in dog Purkinje fibers (Shimizu and Antzelevitch, 2000; Burashnikov and Antzelevitch, 2000). Cordeiro et al. (1998) found that I_{ks} blockading prolongs APD in isolated rabbit Purkinje fiber myocytes. We found that indapamide even in the presence of isoproterenol, at a concentration of the compound that blocks I_{ks} current in isolated guinea pig myocytes (Turgeon et al., 1994), does not prolong APD and does not elicit early afterdepolarizations in isolated rabbit Purkinje fibers. Our results confirm the absence of effects of I_{ks} blockade on APD/QT (Burashnikov and Antzelevitch, 2000; Lengyel et al., 2001; Varró et al., 2000). Using selective I_{ks} blockers such as chromanol 293B or other blocker, other groups also failed to mimic long QT1 in isolated ventricular myocytes, heart or in isolated papillary muscles or in dogs in vivo (Burashnikov and Antzelevitch, 2000; Lengyel et al., 2001; Varró et al., 2000). One reason why I_{ks} blockade did not increase APD in our study may be that the activation of the I_{ks} current normally occurs at around 0 mV (Lengyel et al., 2001) and, thus, voltage is

more positive than the normal Purkinje fiber action potential plateau voltage in our recording action potential in isolated rabbit Purkinje fibers (Fig. 1). Another reason is that the effects of I_{ks} blocking activities may be seen in tachycardic conditions (stimulation rate at 2 or 3 Hz) rather than in a normal rhythm (1 Hz) or in bradycardic conditions. Or, there may be little or no functional I_{ks} currents in isolated Purkinje fibers, although the rabbit Purkinje cells have very small I_{ks} currents (Cordeiro et al., 1998). Our results also confirm the absence of effects of the I_{ks} blocker to mimic long QT1 in other studies in vitro (Burashnikov and Antzelevitch, 2000; Lengyel et al., 2001; Varró et al., 2000).

Two other drug targets are I_{kr} and I_{Na} responsible for the congenital and acquired forms of the long QT2 and long QT3 syndrome. Drugs inhibiting I_{kr} current can be used to mimic long QT2, known to induce prolongation of APD and QT interval, and to elicit early afterdepolarizations arising from plateau and cardiac arrhythmias such as Torsades de Pointes (Abrahamsson et al., 1996; Keating, 1995; Vincent, 1998; Priori et al., 1996, 1997; Shimizu and Antzelevitch, 2000; Studenik et al., 2001; Lu et al., 2001). Inhibition of I_{kr} is the most important mechanism and the common type in the acquired long QT. Similarly, modification of I_{Na} current with anthopleurin, mimicking long QT3, slows the rate of the channel inactivation, prolongs APD, associated with early afterdepolarizations and QT interval associated with Torsades de Pointes (Shimizu and Antzelevitch, 1997, 2000). In the present study, both anthopleurin and dofetilide prolonged APD_{90}, increased triangulation and elicited early afterdepolarizations. These results confirmed that either a reduced I_{kr} or a persistent inward I_{Na} can mimic the classic phenotypic alteration of long QT2 and QT3, resulting clinically in QT prolongation and induction of Torsades de Pointes (Shimizu and Antzelevitch, 1997, 2000; Lu et al., 2000; Antzelevitch, 2001; Rosen, 2001).

The prolongation of APD is an essential prerequisite for the induction of EADs. It has been proposed that early afterdepolarizations constitute a mechanism responsible for Torsades de Pointes in patients with long-QT syndromes (Schechter et al., 1984; Rosen, 2001) as well as in animal models in which QT lengthening drugs are applied (Adamantidis et al., 1998; Priori et al., 1997; Rosen, 2001). Our results show that the prolongation of APD_{90} but not that of APD_{40} is related to the induction of early afterdepolarizations. This may explain that there is a no relationship between the prolongation of APD_{40} and the incidence of early afterdepolarizations, but there is a relationship between the prolongation of APD_{90} and the incidence of early afterdepolarizations in long QT2 and long QT3 models in the isolated rabbit Purkinje fibers (Fig. 3). Our results also confirm the strong relationship between the prolongation of APD_{90} and increase in triangulation (Hondeghem et al., 2001). Similar to the induction of early afterdepolarizations, increased triangulation is not correlated to the changes in APD_{40} in long QT2 and long QT3 models in the rabbit Purkinje fibers (Fig. 3). Furthermore, the extent of the prolongation of the triangulation was greater than that of the prolongation of APD_{90}. This finding confirms that triangulation may be an additional parameter in drug-induced long QTs. Our findings suggest that an increase in triangulation, indicative of changes in the slope of repolarization in phases two to three of the action potential, rather than changes in APD_{40} or APD_{90} alone, determines the potential development of drug-induced early afterdepolarizations. Our findings confirm that triangulation may be an additional parameter in drug-induced long QTs, shown in the isolated rabbit heart (Hondeghem et al., 2001).

Cardio-electrophysiological measurements in vivo and in vitro including in isolated cells or cell lines transected with a specific channel and isolated cardiac tissues are recommended for detecting the potential for QT prolongation and proarrhythmias by a noncardiovascular new chemical entity (NCE) (De Clerck et al., 2002; Haverkamp et al., 2000; Anonymous, 1997). Each individual test has some advantages and disadvantages. Furthermore, the relevance of such single test including the present test to human situation is sometimes not very clear. The isolated rabbit Purkinje fiber only provides a useful tool for understanding the proarrhythmic potential of a drug, associated with long QT and information to understand the mechanisms of drug action and of potential for clinical situation (acquired long-QT syndrome).

In conclusion, our present study indicates that the rabbit Purkinje fibers can be used to detect drugs mimicking long QT2 and QT3 syndrome models, and shows that both prolongation of APD_{90} and of triangulation are important for the induction of early afterdepolarizations in the long QT2 and QT3 models in the rabbit Purkinje fibers. Considering that the most common mechanism for drug-induced long QT is via blocking I_{kr} currents (dofetilide mimicking long QT2), tests on isolated rabbit Purkinje fibers can be used successfully to detect drug-induced long-QT syndrome.

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References

Abrahamsson, C., Carlsson, L., Duker, G., 1996. Lidocaine and nisoldipine attenuate almokalant-induced dispersion of repolarization and early afterdepolarizations in vitro. J. Cardiovasc. Electrophysiol. 7, 1074–1081.

Adamantidis, M.M., Lacroix, D.L., Caron, J.F., Dupuis, B.A., 1995. Electrophysiological and arrhythmogenic effects of the histamine type1-receptor antagonist astemizole on rabbit Purkinje fibers: clinical relevance. J. Cardiovasc. Pharmacol. 26, 319–327.

Adamantidis, M.M., Dumotier, B.M., Caron, J.F., Bordet, R., 1998. Sparfloxacin but not levofloxacin or ofloxacin prolongs cardiac repolarization in rabbit Purkinje fibers. Fundam. Clin. Pharmacol. 12 (1), 70–76.

Anonymous, 1997. Points to consider: the assessment of the potential for QT interval prolongation by non-cardiovascular medicinal products. Publication no. CPMP 986–996. Committee for Proprietary Medicinal Products (CPMP) Human Medicines Evaluation Unit, London, UK.

Antzelevitch, C., 2001. Heterogeneity of cellular repolarization in LQTs: the role of M cells. Eur. Heart J. 3 (Suppl. K), K2–K16.

Burashnikov, A., Antzelevitch, C., 2000. Block of I_{ks} does not induce early afterdepolarization activity but promotes β-adrenergic agonist-induced delayed afterdepolarization activity. J. Cardiovasc. Electrophysiol. 11, 458–465.

Carlsson, L., Amos, G.J., Andersson, B., et al., 1997. Electrophysiological characterization of the prokinetic agents cisapride and mosapride in vivo and in vitro: implications for proarrhythmic potential? J. Pharmacol. Exp. Ther. 282, 220–227.

Chiang, C.E., Roden, D.M., 2000. The long QT syndromes: genetic basis and clinical implications. J. Am. Cardiol. 36, 1–12.

Cordeiro, J.M., Spitzer, K.W., Giles, W.R., 1998. Repolarising K^+ currents in rabbit heart Purkinje fiber cells. J. Physiol. 508 (Pt. 3), 811–823.

Damiano, B.P., Rosen, M.R., 1984. Effects of pacing on triggered activity induced by early afterdepolarizations. Circulation 69, 1013–1025.

De Clerck, F., Van de Water, A., D'Aubioul, J., Lu, H.R., van Roosem, 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.

Haverkamp, W., Breithardt, G., Camm, A.J., Janse, M.J., Rosen, M.R., Antzelevitch, C., Escande, D., Franz, M., Malik, M., Moss, A., Shah, R., 2000. The potential for QT prolongation and proarrhythmia by non-antiarrhythmic drugs: clinical and regulatory implications. Eur. Heart J. 21, 1216–1231.

Hondeghem, L.M., Dujardin, K., De Clerck, F., 2001. Phase 2 prolongation, in the absence of instability and triangulation, antagonizes class III proarrhythmia. Cardiovasc. Res. 50, 345–353.

January, C.T., Chau, V., Makielski, J.C., 1991. Triggered activity in the heart: cellular mechanisms of early after-depolarizations. Eur. Heart J. 12 (Suppl. F), 4–9.

Keating, M.T., 1995. Genetic approaches to cardiovascular disease, supralvulvar aortic stenosis, Williams syndromes, and long-QT syndrome. Circulation 92, 142–147.

Lengyel, C., Lost, N., Virág, L., Varro, A., Lathrop, D.A., Papp, J.G., 2001. Pharmacological block of the slow component of the outward delayed rectifier current (I_{ks}) fails to lengthen rabbit ventricular muscle QTc and action potential duration. Br. J. Pharmacol. 32, 101–110.

Lu, H.R., Mariën, R., Saels, A., De Clerck, F., 2000. Are there sex specific differences in ventricular repolarization or in drug-induced early afterdepolarizations in isolated rabbit Purkinje fibers? Cardiovasc. Pharmacol. 36, 132–139.

Lu, H.R., Mariën, R., Saels, A., De Clerck, F., 2001. Species plays an important role in drug-induced prolongation of action potential duration and early afterdepolarizations in isolated Purkinje fibers. J. Cardiovasc. Electrophysiol. 12, 93–102.

Priori, S.G., Napolitano, C., Cantu, F., Mckenna, W.J., Roden, D.M., Rudy, Y., Schwartz, K., Schwartz, P.J., 1996. Differential response to Na^+ channel blockade, beta-adrenergic stimulation, and rapid pacing in a cellular model mimicking the SCN5A and HERG defects present in the long-QT syndrome. Circ. Res. 78 (6), 1009–1015.

Priori, S.G., Napolitano, C., Paganini, V., Cantu, F., Schwartz, P.J., 1997. Molecular biology of the long QT syndrome: impact on management. Pacing Clin. Electrophysiol. 20 (8), 2052–2057.

Priori, S.G., Barhanin, J., Hauer, R.N., Haverkamp, W., Jongsma, H.J., Kleber, A.G., 1999. Genetic and molecular basis of cardiac arrhythmias: impact on clinical management parts I and II. Circulation 99, 518–528.

Rosen, M.R., 2001. Isolated tissue models and proarrhythmia. Eur. Heart J. 3 (Suppl. K), K64–K69.

Schechter, E., Freeman, C.C., Lazzara, R., 1984. Afterdepolarizations as a mechanism for the long QT syndrome: electrophysiologic studies of a case. J. Am. Coll. Cardiol. 3, 1556–1561.

Shimizu, W., Antzelevitch, C., 1997. Sodium channel block with mexiletine is effective in reducing dispersion of repolarization and preventing Torsade de Pointes in LQT2 and LQT3 models of the long-QT syndrome. Circulation 96, 2038–2047.

Shimizu, W., Antzelevitch, C., 2000. Differential effects of beta-adrenergic agonists and antagonists in LQT1, LQT2 and LQT3 models of the long QT syndrome. J. Am. Coll. Cardiol. 35, 778–786.

Studenik, C.R., Zhou, Z., January, C.T., 2001. Differences in action potential and early afterdepolarization properties in LQT2 and LQT3 models of long QT syndrome. Br. J. Pharmacol. 132, 85–92.

Turgeon, J., Daleau, P., Bennett, B.P., Wiggins, S.S., Selby, L., Roden, D.M., 1994. Block of I_{ks}, the slow component of the delayed rectifier K^+ current, by the diuretic agent indapamide in guinea-pig myocytes. Circ. Res. 75, 879–886.

Varró, A., Baláti, B., Lost, N., Tatscs, J., Virag, L., Lathrop, D.A., Csaba, L., Talosi, L., Papp, J.G., 2000. The role of the delayed rectifier component I_{ks} in dog ventricular muscle and Purkinje fiber repolarization. J. Physiol. 523 (Pt. 1), 67–81.

Vincent, G.M., 1998. The molecular genetics of the long QT syndrome: genes causing fainting and sudden death. Annu. Rev. Med. 49, 263–274.