*Full Paper***QT PRODACT: Evaluation of the Potential of Compounds to Cause QT Interval Prolongation by Action Potential Assays Using Guinea-Pig Papillary Muscles**

Yoshihide Kii^{1,2,*}, Seiji Hayashi^{1,3}, Mitsuyasu Tabo^{1,4}, Takashi Shimosato^{1,5}, Hitoshi Fukuda^{1,6}, Tetsuji Itoh^{1,7}, Hideto Amano^{1,8}, Mamoru Saito^{1,9}, Hajime Morimoto^{1,10}, Kiyoshi Yamada^{1,11}, Atsuhiko Kanda^{1,12}, Toshimasa Ishitsuka^{1,13}, Takanobu Yamazaki^{1,14}, Yoichi Kiuchi^{1,15}, Shinya Taniguchi^{1,16}, Tatsuya Mori^{1,17}, Shigekazu Shimizu^{1,18}, Yuji Tsurubuchi^{1,19}, Shun-ichi Yasuda^{1,20}, Shin-ichi Kitani^{1,21}, Chiaki Shimada^{1,22}, Kazuo Kobayashi^{1,23}, Masaharu Komeno^{1,24}, Chieko Kasai^{1,25}, Toshiyasu Hombo^{1,26}, and Keiji Yamamoto^{1,27}

¹Japan Pharmaceutical Manufacturers Association (JPMA)/QT PRODACT, Tokyo 103-0023, Japan

²Safety Research Laboratories, Dainippon Sumitomo Pharma Co., Ltd., Osaka 564-0053, Japan

³Discovery Research Laboratories, Nippon Shinyaku Co., Ltd., Kyoto 601-8550, Japan

⁴Safety Assessment Department, Chugai Pharmaceutical Co., Ltd., Shizuoka 412-8513, Japan

⁵Research Department, NISSEI BILIS Co., Ltd., Shiga 528-0052, Japan

⁶Research Laboratories, Toyama Chemical Co., Ltd., Toyama 930-8508, Japan

⁷Developmental Research Laboratories, Shionogi & Co., Ltd., Osaka 561-0825, Japan

⁸Kashima Laboratory, Mitsubishi Chemical Safety Institute Ltd., Ibaraki 314-0255, Japan

⁹Drug Safety Research Laboratories, Eisai Co., Ltd., Ibaraki 300-2635, Japan

¹⁰Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Tokushima 771-0192, Japan

¹¹Pharmaceutical Research Laboratories, Toray Industries, Inc., Kanagawa 248-8555, Japan

¹²Pharmacobioregulation Research Laboratory, Taiho Pharmaceutical Co., Ltd., Tokushima 771-0194, Japan

¹³Toxicology Laboratory, Mitsubishi Pharma Corporation, Chiba 292-0818, Japan

¹⁴Research Center, Kyorin Pharmaceutical Co., Ltd., Tochigi 329-0114, Japan

¹⁵Medicinal Development Research Laboratories, Taisho Pharmaceutical Co., Ltd., Saitama 331-9530, Japan

¹⁶Pharmaceutical Research Laboratories, Ajinomoto Co., Inc., Kanagawa 210-8681, Japan

¹⁷Toxicological Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Shizuoka 411-8731, Japan

¹⁸Kobuchisawa Laboratories, Fuji Biomedix Co., Ltd., Yamanashi 408-0044, Japan

¹⁹Pharmacological Research Laboratories, Drug Safety Testing Center Co., Ltd., Saitama 355-0071, Japan

²⁰Study Department, Nihon Bioresearch Inc., Gifu 501-6251, Japan

²¹Pharmacology & Toxicology Department, Ina Research, Inc., Nagano 399-4501, Japan

²²Research and Development Center, Fuso Pharmaceutical Industries, Ltd., Osaka 536-8523, Japan

²³Pharmacodynamics Research Laboratories, Sankyo Co., Ltd., Tokyo 140-8710, Japan

²⁴Fukui Safety Research Laboratories, Ono Pharmaceutical Co., Ltd., Fukui 913-8538, Japan

²⁵Drug Safety Research, Astellas Pharma Inc., Tokyo 174-8511, Japan

²⁶Drug Safety Research, Astellas Pharma Inc., Osaka 532-8514, Japan

²⁷Development Research Center, Takeda Pharmaceutical Co., Ltd., Osaka 532-8686, Japan

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*Corresponding author (affiliation #2). FAX: +81-6-6338-7656
E-mail: yoshihide-kii@ds-pharma.co.jp

Abstract. Certain compounds that prolong QT interval in humans have little or no effect on action-potential (AP) duration used traditionally, but they inhibit rapidly-activated-delayed-rectifier potassium currents (I_{Kr}) and/or human ether-a-go-go-related gene (hERG) currents. In this study using isolated guinea-pig papillary muscles, we investigated whether new parameters in AP assays can detect the inhibitory effects of various compounds on I_{Kr} and/or hERG currents with high sensitivity. The difference in AP duration between 60% and 30% repolarization, 90% and 60% repolarization, and 90% and 30% repolarization (APD_{30-60} , APD_{60-90} , and APD_{30-90} , respectively) were calculated as the new parameters. All the 15 I_{Kr} and/or hERG current inhibitors that have been reported (9 compounds) or not reported (6 compounds) to inhibit calcium currents prolonged APD_{30-60} , APD_{60-90} , and/or APD_{30-90} ; and 8 of the 15 inhibitors prolonged APD_{30-60} , APD_{60-90} , and/or APD_{30-90} more potently than APD_{90} . The APD_{30-60} , APD_{60-90} , and APD_{30-90} measurements revealed no difference in sensitivity when evaluating the effects of the I_{Kr} and/or hERG current inhibitors on the three parameters. On the other hand, compounds with little or no effect on hERG currents had no effect on APD_{30-60} , APD_{60-90} , or APD_{30-90} . Therefore, it is concluded that in AP assays using isolated guinea-pig papillary muscles, APD_{30-60} , APD_{60-90} , and APD_{30-90} are useful indexes for evaluating the inhibitory effects of compounds including mixed ion-channel blockers on I_{Kr} and/or hERG currents. Supplementary material (Appendix): available only at <http://dx.doi.org/10.1254/jphs.QT-C8>

Keywords: action-potential duration (APD) between 30% and 60% repolarization (APD_{30-60}), APD_{60-90} , APD_{30-90} , human ether-a-go-go-related gene (hERG) current, QT PRODACT

Introduction

Many non-cardiac drugs have recently been shown to prolong electrocardiographic QT interval, leading to life-threatening ventricular arrhythmia, torsade de pointes (TdP) in humans (1, 2). Therefore, the S7B guidelines of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use recommend the conducting of in vitro I_{Kr} assay and in vivo QT assays to evaluate the potential of compounds to cause delayed ventricular repolarization (QT interval prolongation) (3). It is well known that QT interval correlates with action-potential (AP) duration in ventricular muscles and Purkinje fibers. Accordingly, AP assays using isolated ventricular muscles and Purkinje fibers have been used to evaluate the potential of compounds to cause QT interval prolongation. AP is composed of various inward and outward ion currents such as sodium, calcium, and potassium currents (Fig. 1); and the effects of a compound on AP parameters can predict its effects on each ion current. In isolated guinea-pig papillary muscles, astemizole and terfenadine, two second generation antihistamines, and pimozone, an antipsychotic agent, have been reported to have little or no effect on AP duration at 90% repolarization (APD_{90}), a parameter traditionally used in AP assays (4). However, these drugs have been shown to prolong QT interval in humans and inhibit rapidly-activated-delayed-rectifier potassium currents (I_{Kr}) and/or I_{Kr} -like human ether-a-

go-go-related gene (hERG) currents (1). These results indicate that APD_{90} alone is not reliable enough for evaluating the inhibitory effects of compounds on I_{Kr} and/or hERG currents.

In isolated rabbit hearts, many I_{Kr} and/or hERG current inhibitors have been shown to prolong monophasic AP duration between 30% to 90% repolarization but not between 10% to 30% repolarization (5, 6). Furthermore, it has been reported that dofetilide, a potent hERG current inhibitor (7, 8), prolongs not only AP duration but also the difference between AP duration at 90% repolarization and that at 40% repolarization in isolated rabbit Purkinje fibers (9). These results suggest that determining whether a compound prolongs the difference in AP duration between fixed points of repolarization can help predict its inhibitory effects on I_{Kr} and/or hERG currents and consequently its potential for QT interval prolongation. It has been reported that nifedipine, a potent calcium channel blocker, reduces APD-prolongation induced by dofetilide. This finding suggests that inhibition of inward calcium current (I_{Ca}) attenuates APD-prolongation induced by I_{Kr} and/or hERG current inhibitors. Recently, a new mathematical model of membrane excitation, the Kyoto Model, has been proposed to clarify the role of various ion channels in membrane excitability in ventricular cells (10). Using the Kyoto Model, we calculated major ionic currents contributing to the formation of AP in ventricular cells electrically driven at 1.0 Hz (Table 1) and showed that the contribution of I_{Ca} in APD_{60-90} formation is

Fig. 1. Typical patterns of action potential in ventricular cells (upper) and major ionic currents contributing to formation of the action potential (lower). Upper: V_m : membrane potential of action potential in ventricular cells. Lower: Positive and negative values in the vertical bar represent outward and inward ion currents, respectively.

much lower than that in APD_{30-60} or APD_{30-90} and that APD_{60-90} is a suitable AP parameter to detect inhibition of hERG currents by I_{Kr} and/or hERG current inhibitors.

Using isolated guinea-pig papillary muscles, we investigated in this study whether new parameters in AP assays can detect the inhibitory effects of various compounds on I_{Kr} and/or hERG currents with high sensitivity. The differences in AP duration between 60% and 30% repolarization, 90% and 60% repolarization, as well as 90% and 30% repolarization (APD_{30-60} , APD_{60-90} , and APD_{30-90} , respectively) were calculated as the new parameters. However, it is worth noting here that a compound's potential for QT interval prolongation in humans should be assessed by integrating non-clinical, clinical, and other relevant information. It is, therefore, difficult to evaluate the safety of each compound in humans based only on the results of this study.

Materials and Methods

Electrophysiology

This study was performed in accordance with the Guidelines for the Care and Use of Animals defined by the Internal Committee of Experimental Animals in each organization given in the list of authors' affiliations for this paper.

Details of AP assays are described in another paper in this issue (4). Briefly, papillary muscles were excised from the right ventricles of male Hartley guinea pigs and placed in an organ bath. The preparations were superfused with Tyrode's solution ($37.0 \pm 0.5^\circ\text{C}$) equilibrated with a gas mixture of 95% O_2 and 5% CO_2 at a flow rate of 5 to 10 mL/min. The preparations were then electrically stimulated by an electrical stimulator via an isolation unit at a frequency of 1 Hz with

Table 1. Calculated major ionic current areas on simulated action potential waveform of ventricular myocytes using the Kyoto Model

	I_{Na}	I_{Ca}	I_{Kr}	I_{Ks}
APD_{30}	-14237	-10624	381	2706
APD_{60}	-15495	-13773	1297	4261
APD_{90}	-15884	-13909	1590	4333
APD_{30-60}	-1259	-3148	916	1555
APD_{60-90}	-388	-136	293	71
APD_{30-90}	-1647	-3284	1209	1626

Each value shows the total ionic current area ($\text{pA} \times \text{ms}$) calculated by the equations shown in reference [10]. Simulation conditions were as follows: extracellular sodium, potassium, and calcium concentrations were 152.4, 4, and 2 mmol/L, respectively. Stimulation and sampling frequencies were 1.0 Hz and 0.01 ms, respectively. Initial values were as follows: intracellular sodium, potassium, calcium, and adenosine 5'-triphosphate concentrations were 6.33, 141.75, 2.06×10^{-5} , and 4.48 mmol/L, respectively. Membrane potential was set at -94.05 mV. A negative value shows an inward ionic current. Note that current area for I_{Ca} and I_{Ks} in APD_{60-90} is much lower than that in APD_{30-60} or APD_{30-90} and that the ratio of I_{Kr}/I_{Ks} in APD_{60-90} is higher than that in APD_{30-60} or APD_{30-90} .

Table 2. Effects of I_{Kr} and/or hERG current inhibitors reported to inhibit I_{Ca} on APD_{30-60} , APD_{60-90} , and APD_{30-90} in isolated guinea-pig papillary muscles

Compound	Concentration ($\mu\text{mol/L}$)	APD_{30-60}	APD_{60-90}	APD_{30-90}
Bepridil (Antianginal agent)	0.1	3 ± 1	1 ± 1	2 ± 1
	1	5 ± 1	4 ± 1	4 ± 1
	10	$18 \pm 3^{**}$	$20 \pm 3^{**}$	$19 \pm 3^{**}$
	100	$26 \pm 3^{**}$	$34 \pm 3^{**}$	$29 \pm 2^{**}$
Cisapride (Gastroprokinetic agent)	0.01	3 ± 1	-2 ± 2	2 ± 1
	0.1	$15 \pm 2^{**}$	5 ± 3	$12 \pm 2^{**}$
	1	$44 \pm 4^{**}$	$25 \pm 3^{**}$	$38 \pm 3^{**}$
Disopyramide (Antiarrhythmic (Ia))	1	11 ± 2	4 ± 1	9 ± 2
	3	$27 \pm 4^{**}$	$9 \pm 2^*$	$21 \pm 3^{**}$
	30	$35 \pm 6^{**}$	$38 \pm 4^{**}$	$36 \pm 4^{**}$
Flecainide (Antiarrhythmic (Ic))	1	0 ± 4	0 ± 2	0 ± 3
	3	$8 \pm 1^*$	4 ± 1	$7 \pm 1^*$
	10	$16 \pm 3^{**}$	$13 \pm 3^{**}$	$15 \pm 2^{**}$
Haloperidol (Antipsychotic)	0.1	5 ± 1	-3 ± 1	3 ± 1
	1	14 ± 1	1 ± 1	9 ± 1
	10	$48 \pm 12^{**}$	$32 \pm 5^{**}$	$43 \pm 9^{**}$
Pimozide (Antipsychotic)	0.1	4 ± 1	2 ± 1	3 ± 1
	1	5 ± 1	1 ± 1	4 ± 1
	10	$11 \pm 2^{**}$	$6 \pm 1^{**}$	$9 \pm 2^{**}$
Quinidine (Antiarrhythmic (Ia))	1	6 ± 3	-3 ± 3	3 ± 3
	10	$40 \pm 5^{**}$	$25 \pm 4^{**}$	$35 \pm 4^{**}$
	100	$30 \pm 4^{**}$	$56 \pm 5^{**}$	$39 \pm 2^{**}$
Terfenadine (Antihistamine)	3	-2 ± 2	-1 ± 1	-2 ± 1
	10	4 ± 2	0 ± 2	3 ± 1
	20	0 ± 1	$6 \pm 2^*$	2 ± 1
Verapamil (Antihypertensive agent)	1	$16 \pm 3^{**}$	7 ± 1	$13 \pm 2^{**}$
	10	$35 \pm 3^{**}$	$27 \pm 3^{**}$	$33 \pm 3^{**}$
	100	$12 \pm 3^{**}$	$73 \pm 6^{**}$	$30 \pm 3^{**}$

Data are expressed as the percent change from the pre-treatment value. Each value represents the mean $\pm$ S.E.M. of 6 experiments. * $P < 0.05$, ** $P < 0.01$ vs vehicle by parametric Dunnett's test.

rectangular pulses of 1-ms duration whose voltage was 1.5–2.5 times higher than the diastolic threshold. A glass microelectrode (10–40 M Ω) filled with 3 mol/L KCl was used to measure transmembrane APs. The APs were amplified with an amplifier and recorded by an AP recording/analysis system for a 30-min application period.

Test compounds

The 21 test compounds used in this study were divided into three groups: 1) I_{Kr} and/or hERG current inhibitors reported to inhibit calcium currents: bepridil (11), cisapride (12), disopyramide (13), flecainide (14),

haloperidol (15), pimozide (16), quinidine (14, 17), terfenadine (18, 19), and verapamil (20); 2) I_{Kr} and/or hERG current inhibitors not reported to inhibit calcium currents (21): astemizole, ciprofloxacin, E-4031, MK-499, *dl*-propranolol, and thioridazine; and 3) other compounds with little or no effect on hERG currents (21): amoxicillin, aspirin, captopril, diphenhydramine, lidocaine, and nifedipine.

Data analysis

APD_{30-60} , APD_{60-90} , and APD_{30-90} were calculated as the differences in AP duration between 60% and 30%, 90% and 60%, and 90% and 30% repolarization, respec-

Table 3. Effects of I_{Kr} and/or hERG current inhibitors not reported to inhibit I_{Ca} on APD_{30-60} , APD_{60-90} , and APD_{30-90} in isolated guinea-pig papillary muscles

Compound	Concentration ($\mu\text{mol/L}$)	APD_{30-60}	APD_{60-90}	APD_{30-90}
Astemizole (Antihistamine)	0.1	2 ± 1	-2 ± 0	1 ± 0
	1	$13 \pm 3^{**}$	$3 \pm 2^*$	$10 \pm 2^{**}$
	10	$19 \pm 3^{**}$	$11 \pm 3^{**}$	$16 \pm 3^{**}$
Ciprofloxacin (Antimicrobial agent)	1	-1 ± 0	0 ± 1	-1 ± 0
	10	1 ± 0	0 ± 1	1 ± 0
	100	$6 \pm 1^{**}$	$6 \pm 1^{**}$	$6 \pm 1^{**}$
E-4031 (Antiarrhythmic (III))	0.001	3 ± 1	0 ± 1	2 ± 1
	0.01	$16 \pm 2^*$	$6 \pm 2^*$	$13 \pm 2^*$
	0.1	$71 \pm 7^{**}$	$31 \pm 4^{**}$	$59 \pm 6^{**}$
MK-499 (Antiarrhythmic (III))	0.01	10 ± 3	1 ± 2	7 ± 3
	0.1	$25 \pm 4^{**}$	$10 \pm 2^*$	$20 \pm 3^{**}$
	1	$58 \pm 5^{**}$	$30 \pm 4^{**}$	$49 \pm 5^{**}$
<i>dl</i> -Propranolol (Antiarrhythmic (II))	0.3	0 ± 2	-3 ± 2	-1 ± 2
	3	2 ± 1	6 ± 2	3 ± 1
	30	-2 ± 3	$34 \pm 4^{**}$	$9 \pm 3^{**}$
Thioridazine (Antipsychotic)	0.1	5 ± 1	2 ± 2	4 ± 1
	1	$10 \pm 1^*$	4 ± 2	8 ± 1
	10	$32 \pm 5^{**}$	$38 \pm 7^{**}$	$34 \pm 6^{**}$

Data are expressed as the percent change from the pre-treatment value. Each value represents the mean $\pm$ S.E.M. of 6 experiments. * $P < 0.05$, ** $P < 0.01$ vs vehicle by parametric Dunnett's test.

tively. Percent change in AP duration from the pre-treatment value in each test-compound-treated group was compared to that in the vehicle-control group by the parametric Dunnett's test (2-tailed). Data are expressed as the mean $\pm$ S.E.M. Statistical significance was defined as $P < 0.05$.

Results

Absolute values of APD_{30-60} , APD_{60-90} , and APD_{30-90} before and 30 min after perfusion of vehicle or test compounds are shown in Appendices 1-1 to 3-2 as Supplemental Materials with this article online.

Effects of I_{Kr} and/or hERG current inhibitors reported to inhibit calcium currents on APD_{30-60} , APD_{60-90} , and APD_{30-90} (Table 2, Appendices 1-1 to 1-3)

Bepidil (10 and 100 $\mu\text{mol/L}$), disopyramide (3 and 30 $\mu\text{mol/L}$), haloperidol (10 $\mu\text{mol/L}$), pimozide (10 $\mu\text{mol/L}$), and quinidine (10 and 100 $\mu\text{mol/L}$) prolonged APD_{30-60} , APD_{60-90} , and APD_{30-90} . Cisapride at 0.1 $\mu\text{mol/L}$ prolonged APD_{30-60} and APD_{30-90} without affecting APD_{60-90} , whereas at 1 $\mu\text{mol/L}$, it prolonged APD_{30-60} , APD_{60-90} , and APD_{30-90} . Terfenadine at the highest

concentration of 20 $\mu\text{mol/L}$ slightly prolonged APD_{60-90} without affecting APD_{30-60} or APD_{30-90} . Flecainide (10 $\mu\text{mol/L}$) and verapamil (10 and 100 $\mu\text{mol/L}$) prolonged APD_{30-60} , APD_{60-90} , and APD_{30-90} , while flecainide at 3 $\mu\text{mol/L}$ and verapamil at 1 $\mu\text{mol/L}$ prolonged APD_{30-60} and APD_{30-90} without affecting APD_{60-90} .

Effects of I_{Kr} and/or hERG current inhibitors not reported to inhibit calcium currents on APD_{30-60} , APD_{60-90} , and APD_{30-90} (Table 3, Appendices 2-1 and 2-2)

Astemizole (1 and 10 $\mu\text{mol/L}$), ciprofloxacin (100 $\mu\text{mol/L}$), E-4031 (0.01 and 0.1 $\mu\text{mol/L}$), and MK-499 (0.1 and 1 $\mu\text{mol/L}$) prolonged APD_{30-60} , APD_{60-90} , and APD_{30-90} . *dl*-Propranolol at the highest concentration of 30 $\mu\text{mol/L}$ prolonged APD_{60-90} and APD_{30-90} without affecting APD_{30-60} . Thioridazine at 1 $\mu\text{mol/L}$ prolonged APD_{30-60} without affecting APD_{60-90} or APD_{30-90} , whereas at the 10- $\mu\text{mol/L}$ concentration, it prolonged APD_{30-60} , APD_{60-90} , and APD_{30-90} .

Effects of compounds with little or no effect on hERG currents on APD_{30-60} , APD_{60-90} , and APD_{30-90} (Table 4, Appendices 3-1 and 3-2)

Amoxicillin, aspirin, and captopril had no effect on

Table 4. Effects of the compounds with little or no effect on I_{Kr} and/or hERG current on APD₃₀₋₆₀, APD₆₀₋₉₀, and APD₃₀₋₉₀ in isolated guinea-pig papillary muscles

Compound	Concentration ($\mu\text{mol/L}$)	APD ₃₀₋₆₀	APD ₆₀₋₉₀	APD ₃₀₋₉₀
Amoxicillin (Antimicrobial agent)	27	1 ± 1	-3 ± 1	-1 ± 1
	270	1 ± 2	0 ± 2	0 ± 2
	2700	3 ± 1	3 ± 1	3 ± 1
Aspirin (Antiinflammatory)	100	-6 ± 2	-6 ± 2	-6 ± 2
	1000	5 ± 5	1 ± 3	4 ± 5
	10000	-1 ± 4	-2 ± 3	-2 ± 2
Captopril (Antihypertensive /antianginal agent)	1	0 ± 1	-1 ± 2	0 ± 1
	10	1 ± 1	2 ± 1	1 ± 1
	100	-1 ± 1	0 ± 0	-1 ± 1
Diphenhydramine (Antihistamine)	0.3	4 ± 2	-1 ± 1	2 ± 2
	3	11 ± 2	4 ± 4	$8 \pm 1^*$
	30	-7 ± 4	$32 \pm 3^{**}$	5 ± 4
Lidocaine (Antiarrhythmic (Ib))	1	-2 ± 3	-1 ± 1	-2 ± 2
	10	-2 ± 2	-2 ± 2	-2 ± 1
	100	$-10 \pm 4^*$	11 ± 6	-3 ± 5
Nifedipine (Antihypertensive agent)	0.3	-1 ± 3	0 ± 1	-1 ± 3
	3	-4 ± 5	-4 ± 2	-4 ± 4
	30	$-13 \pm 5^*$	-2 ± 2	-10 ± 4

Data are expressed as the percent change from the pre-treatment value. Each value represents the mean $\pm$ S.E.M. of 6 experiments. * $P < 0.05$, ** $P < 0.01$ vs vehicle by parametric Dunnett's test.

APD₃₀₋₆₀, APD₆₀₋₉₀, or APD₃₀₋₉₀. Diphenhydramine at 3 and 30 $\mu\text{mol/L}$ prolonged APD₃₀₋₉₀ and APD₆₀₋₉₀, respectively, without affecting APD₃₀₋₆₀. Lidocaine and nifedipine shortened APD₃₀₋₆₀ without affecting APD₆₀₋₉₀ or APD₃₀₋₉₀.

Discussion

Of the 15 I_{Kr} and/or hERG current inhibitors tested in this study, astemizole, bepridil, cisapride, disopyramide, E-4031, flecainide, haloperidol, MK-499, quinidine, and thioridazine prolonged APD₃₀₋₆₀, APD₆₀₋₉₀, and APD₃₀₋₉₀ by more than 10% of each pre-treatment value in isolated guinea-pig papillary muscles. These 10 inhibitors also prolonged APD₉₀ (4), although astemizole and bepridil only slightly prolonged APD₉₀. Pimozide, *dl*-propranolol, terfenadine, and verapamil prolonged APD₃₀₋₆₀, APD₃₀₋₆₀, and/or APD₃₀₋₆₀. However, these four I_{Kr} and/or hERG current inhibitors did not prolong APD₉₀ (4). Diphenhydramine, a weak I_{Kr} blocker (22), prolonged APD₆₀₋₉₀ and APD₃₀₋₉₀ but did not affect APD₉₀ (4). Amoxicillin, aspirin, captopril, lidocaine, and nifedipine did not prolong APD₃₀₋₆₀, APD₆₀₋₉₀, or APD₃₀₋₉₀. It is well known that amoxicillin, aspirin,

captopril, lidocaine, and nifedipine have little or no inhibitory effect on I_{Kr} and/or hERG currents (21). These results indicate that in isolated guinea-pig papillary muscles, the sensitivity of APD₃₀₋₆₀, APD₆₀₋₉₀, and APD₃₀₋₉₀ in detecting the inhibitory effects of compounds on I_{Kr} and/or hERG currents is higher than that of APD₉₀.

All the 15 I_{Kr} and/or hERG current inhibitors prolonged APD₆₀₋₉₀ by more than 5% of each pre-treatment value (Table 5), while 14 of these inhibitors (terfenadine excluded) prolonged APD₃₀₋₉₀ by more than 5% of each pre-treatment value. Thirteen I_{Kr} and/or hERG current inhibitors (terfenadine and *dl*-propranolol excluded) prolonged APD₃₀₋₆₀ by more than 5% of each pre-treatment value. Diphenhydramine prolonged APD₆₀₋₉₀ by more than 10% and APD₃₀₋₉₀ by 5% – 10%, although this compound has been reported to moderately inhibit I_{Kr} (22). From our results calculated using the Kyoto Model (10), which is a new mathematical model of membrane excitation, it is suggested that the I_{Ca} contribution in the formation of APD₆₀₋₉₀ is much lower than that in APD₃₀₋₆₀ or APD₃₀₋₉₀. This hypothesis is supported by the fact that nifedipine, a calcium channel blocker, shortened APD₃₀₋₆₀ without affecting APD₆₀₋₉₀.

Table 5. Summary of the effects of I_{Kr} and/or hERG current inhibitors and other compounds on APD_{30-60} , APD_{60-90} , APD_{30-90} , and APD_{90} in isolated guinea-pig papillary muscles

	APD_{30-60}	APD_{60-90}	APD_{30-90}	APD_{90}^a
I_{Kr} and/or hERG current inhibitors reported to inhibit I_{Ca}				
Bepridil	++	++	++	±
Cisapride	++	++	++	++
Disopyramide	++	++	++	++
Haloperidol	++	++	++	++
Pimozide	++	+	+	–
Quinidine	++	++	++	++
Terfenadine	–	+	–	–
Flecainide	++	++	++	+
Verapamil	++	++	++	–
I_{Kr} and/or hERG current inhibitors not reported to inhibit I_{Ca}				
Astemizole	++	++	++	±
E-4031	++	++	++	++
MK-499	++	++	++	++
Thioridazine	++	++	++	++
Ciprofloxacin	+	+	+	±
<i>dl</i> -Propranolol	–	++	+	–
Compounds with little or no effect on hERG current				
Amoxicillin	–	–	–	–
Aspirin	–	–	–	–
Captopril	–	–	–	–
Diphenhydramine	–	++	+	–
Lidocaine	–	–	–	–
Nifedipine	–	–	–	–

–: no prolongation; ±, +, and ++: significant prolongation by less than 5%, 5% – 10%, and more than 10%, respectively, of pre-treatment values. ^a: Data from another report in this issue (Ref. 4).

On the other hand, despite the fact that disopyramide (13), quinidine (14, 17), and pimozide (16) inhibit I_{Ca} , they prolonged APD_{30-60} , APD_{60-90} , and APD_{30-90} . Therefore, it is likely that evaluation of the effects of compounds on APD_{30-60} , APD_{60-90} , and APD_{30-90} can predict the inhibitory effects of mixed ion-channel blockers on I_{Kr} and/or hERG currents, although no difference in sensitivity, when evaluating the inhibitory effects of mixed ion-channel blockers on hERG, can be expected between APD_{30-60} , APD_{60-90} , and APD_{30-90} measurement.

Of the I_{Kr} and/or hERG current inhibitors tested in our study, astemizole (23), cisapride (24), E-4031 (25), flecainide (26), ciprofloxacin (27), pimozide (28), and quinidine (29) have been reported to inhibit I_{Kr} or hERG currents with little or no effect on slowly-activated-delayed-rectifier potassium currents (I_{Ks}) or I_{Ks} -like KvLQT1/mink currents. On the other hand, the prolongation of APD_{30-60} and APD_{30-90} induced by cisapride at 1 μ mol/L was greater than that of APD_{60-90} . Furthermore, the concentrations of flecainide, thioridazine, and

verapamil at which prolongation of APD_{30-60} and/or APD_{30-90} occurred were lower than those at which prolongation of APD_{60-90} was obtained. These findings indicate that when evaluating the inhibitory effects of compounds on hERG currents, measurements of APD_{30-60} , APD_{60-90} , and APD_{30-90} are highly sensitive and reliable.

Astemizole, bepridil, cisapride, haloperidol, terfenadine, and quinidine have been identified as compounds with proarrhythmic potential in the rabbit heart model (6). These findings support our results showing that these six compounds prolong APD_{30-60} , APD_{60-90} , and/or APD_{30-90} . *dl*-Propranolol and verapamil prolonged APD_{30-60} , APD_{60-90} , and/or APD_{30-90} , although both compounds were identified as having no proarrhythmic activity in the rabbit heart model (6). It has been reported that *dl*-propranolol at therapeutic concentrations does not affect hERG currents (30) and that verapamil at therapeutic concentrations does not affect QT interval in humans (1). These results suggest that evaluation of the

effects of test compounds on APD₃₀₋₆₀, APD₆₀₋₉₀, and APD₃₀₋₉₀ identify compounds with little or no proarrhythmic action as false positive compounds. Thus, integration of studies such as in vivo QT assays using conscious and/or anesthetized animals and evaluation of other AP parameters, such as APD₉₀, would improve our assessment of a compound's potential for QT interval prolongation.

The S7B guidelines of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use recommend the conducting of the in vitro I_{Kr} assay (hERG current assay) and in vivo QT assay to evaluate the potential of compounds to cause delayed ventricular repolarization (QT interval prolongation) (3). In addition, the guidelines encourage the enforcement of follow-up studies to detect a compound's potential for causing delayed ventricular repolarization. The results of this study suggest that the AP assay in isolated guinea-pig papillary muscles using APD₃₀₋₆₀, APD₆₀₋₉₀, and APD₃₀₋₉₀ as parameters can help detect a compound's potential for inducing QT interval prolongation. However, despite the fact that *dl*-propranolol and verapamil have not induced excessive QT interval prolongation leading to TdP (1), both compounds prolonged APD₆₀₋₉₀ and APD₃₀₋₉₀. Therefore, the potential of compounds for QT interval prolongation in humans should be assessed by integrating non-clinical, clinical, and other relevant information.

In conclusion, APD₃₀₋₆₀, APD₆₀₋₉₀, and APD₃₀₋₉₀ in AP assays using isolated guinea-pig papillary muscles are useful indexes for evaluating the effects of compounds including mixed ion-channel blockers on I_{Kr} and/or hERG currents.

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