Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.4, No.3, 127-136, 2017

Letter

The utility of human iPS cell-derived cardiomyocytes in predicting the clinical risk of drugs that display discordance of cardiotoxicity by species

Nobuo Aikawa

Translational Research Unit, R&D Division, Kyowa Hakko Kirin Co., Ltd.,
1188, Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka, 411-8731, Japan

(Received June 8, 2017; Accepted June 13, 2017)

ABSTRACT — Drug candidates sometimes cause a prolongation of the electrocardiogram QT-interval (QT) and *torsades de pointes* in humans, despite the fact that they do not cause them in non-rodent animals. Recent studies suggest that the cardiomyocytes derived from human induced-pluripotent stem cells (hiPS-CMs) are of sufficient quality to assess the cardiotoxicity of drugs in the preclinical setting. Thus, the usefulness of hiPS-CMs in correctly predicting the cardiotoxicity of drug candidates in the clinical setting, was examined using conventional drugs in the calcium transient analysis system FDSS/μCELL and the multielectrode array system MED64. The selection of the test drugs was based on previously reported studies. E-4031 and cisapride prolong the QT in humans, dogs and monkeys. Both drugs prolonged the calcium fluorescence peak width (PWD) in the FDSS/μCELL system and the field potential duration (FPD) in the MED64 system, both of which are thought to be surrogates of the QT. Diphenhydramine, famotidine and E-8010 prolong the QT in humans but not in dogs or monkeys. These drugs prolonged the PWD and FPD. On the other hand, verapamil and nifedipine prolong the QT in dogs or monkeys but not in humans. Both drugs shortened the PWD and FPD. These results suggest that the hiPS-CMs assay could correctly predict the QT effects in humans. The hiPS-CMs would be useful for predicting the effects of drug candidates on the QT of humans in preclinical *in vitro* studies.

Key words: Cardiomyocytes, Cardiotoxicity, Multielectrode array, MED64, Ca transient, FDSS/μCELL

INTRODUCTION

More than 80% of drug candidates entered into clinical trials are terminated before they launch (Hay *et al*., 2014). The reason is usually due to a lack of efficacy or safety. With regard to safety, the toxicity information of drug candidates is mainly obtained from animal experiments, but the results of animal experiments do not always reflect human toxicity (Olson *et al*., 2000). The discrepancy in the toxicity of the drug candidates is found, not only between human and experimental animals, but also between experimental animals (Olson *et al*., 2000; Tamaki *et al*., 2013; Vargas *et al*., 2015). These discrepancies in the toxicological response might lead to the selection of inappropriate drug candidates for clinical trials. As a result, many clinical trials are terminated before launch. In order to for the clinical trials of drug candidates to be successful, a new assay is needed to correctly predict human drug toxicity in the preclinical setting.

The target organs of toxicity that most frequently lead to the termination of clinical trials of drug candidates include the cardiovascular system, liver and nervous system (Watkins, 2011). Above all, a well-known cardiac arrhythmia, *torsade de pointes* (Tdp), which represents the most common and severe event, and which can cause sudden death in patients. This event has led to the termination of clinical trials, and the withdrawal of drugs from the market in the past.

The most common mechanism of drug-induced Tdp was found to be the prolongation of the QT-interval (QT) on electrocardiography, due to the inhibition of the human ether-a-go-go related gene (hERG)—a gene coding the alpha subunit of an Ikr channel. In preclinical studies, drug candidates are typically assessed in *in vitro*

Correspondence: Nobuo Aikawa (E-mail: nobuo.aikawa@kyowa-kirin.co.jp)

hERG/Ikr current inhibition experiments and *in vivo* QT prolongation experiments. Because cardiomyocytes beat due to the combined effects of multi-ion channels, pumps and exchangers, drug candidates sometimes prolong the QT without inhibiting the hERG/Ikr current and do not always prolong the QT by inhibiting the hERG/Ikr current (Redfern *et al*., 2003). Moreover, a case was reported in which the risk of human cardiotoxicity was not detected in animals; this may have been caused by the dosage, the experimental conditions or the selection of animal species (Vargus *et al*., 2015). Furthermore, the binding affinity of drugs to the ion channels and the distribution of the ion channels in the heart differ in each species (Peng *et al*., 2010; McNeill, 1984; O'hara and Rudy, 2012; Lu *et al*., 2001). If the induction of human cardiotoxicity by drug candidates is detected using non-rodent models, then the waste—in terms of the time and the resources spent in drug development—will be enormous, because human cardiotoxicity is usually detected in the late stage of preclinical drug development.

Cardiomyocytes derived from human induced pluripotent stem cells (hiPS-CMs) have recently been marketed by several vendors. These hiPS-CMs generate human cardiac ion channels, pumps and exchangers and—needless to say—represent no-problems in terms of species selection or bioethics. An *in vitro* assay using hiPS-CMs is thought to be a suitable tool for assessing the clinical cardiotoxicity of drug candidates. In order to correctly predict the clinical risk of the cardiotoxicity of drug candidates in the early stage of preclinical drug development—before animal experiments are initiated—the hiPS-CMs assay was conducted. In drug development, non-rodent animal experiments are emphasized, but the QT-actions of drug candidates in non-rodent animals are not always in concordance with those in humans. There have been no reports comparing the *in vitro* data of humans and the *in vivo* data of humans and non-rodents in the QT prolongation experiment. In the present study, two different hiPS-CMs assays were conducted using drugs that have been reported to have different effects on the QT-action in non-rodent animals and humans, and the usefulness of the hiPS-CMs assay in clinical prediction was investigated.

MATERIALS AND METHODS

Testing drugs

The test drugs were chosen based on previous reports in the literature. The test concentration of each drug covered a broad range of receptor-inhibitory activity in relation to the hERG/Ikr channel or the main medicinal effect. E-4031 n-hydrate (E-4031) and verapamil hydrochloride (verapamil) were purchased from Wako Pure Chemical (Osaka, Japan). Cisapride monohydrate (cisapride), famotidine and nifedipine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Diphenhydramine hydrochloride (diphenhydramine) was purchased from Tokyo Chemical (Tokyo, Japan), E-8010 was made in-house. All of the drugs were dissolved in dimethylsulfoxide at 1000-fold the highest tested concentration. They were then subdivided and frozen at -30°C until use. In the FDSS/μCELL assay, the frozen drug samples were thawed and reconstituted into sterile distilled water at 10-fold the concentration that was to be applied. In a MED64 assay, frozen drug samples were thawed and reconstituted into dimethylsulfoxide at 1000-fold the concentration that was to be applied. Because a preliminary experiment showed that ≤ 0.5 vol% dimethylasufoxide does not affect the function of hiPS-CMs, the maximal concentration of dimethylsulfoxide in all of the experiments was ≤ 0.5 vol%.

Cells

hiPS-CMs were used (iCell® cardiomyocytes²; Cellar Dynamics International, Madison, WI, USA). The frozen hiPS-CMs were thawed according to manufacturer's instructions and the number of cells was counted using a hemocytometer. The cell density was adjusted with a plating culture medium, seeded to the well of a culture plate or the MED probe, and cultured in a 5% CO_2 incubator at 37°C under humidification. After approximately 3 hr, the medium was replaced with a maintenance culture medium, and the cells were cultured in a 5% CO_2 incubator.

The FDSS/μCELL assay

The FDSS/μCELL system (Hamamatsu Photonics, Shizuoka, Japan) is a high-throughput microplate reader that can analyze the fluorescence intensity, intracellular calcium (Ca) flux, thallium (potassium surrogate) concentration, and membrane potential of cells automatically. The assay was conducted according to the iCell® cradiomyocytes² application protocol of Cellular Dynamics International and a user guide from Hamamatsu Photonics.

The hiPS-CMs were seeded at 3.0×10^4 cells/0.1 mL/well in a 96-well clear bottom black half area cell culture plate (Nippi, Tokyo, Japan) and cultured in a 5% CO_2 incubator (37°C, with humidification). The culture medium was replaced with fresh medium every other day. Eight or 9 days later, the medium was replaced in all wells of the 96-well culture pate at 45 μL/well, and the cells were cultured in a 5% CO_2 incubator for more than 1 hr. A 45 μL/well of Ca indicator (EarlyTox® cardiotox-

icity kit, Molecular Device Japan, Tokyo, Japan) was added to all of the wells with cells in the 96-well culture plate and incubated in 5% CO_2 for 1 hr. The 96-well culture plate was set in the FDSS/μCELL device (Hamamatsu Photonics), and stabilized for 10 min at 37°C in the air. The Ca fluorescence waveform was recorded for 1 min as the baseline value. The drugs and dimethylsulfoxide-vehicle were then applied automatically to the wells of the 96 well culture plate at a volume of 10 μL/well (each concentration was applied to 4 wells). Ten minutes later, the Ca fluorescence waveform was recorded for 1 min.

The MED64 assay

The MED64 system (Alpha Med Scientific, Osaka, Japan) was used as a multielectrode array system. This system is thought to be able to predict arrhythmias with high accuracy (Kitaguchi *et al*., 2016; Nozaki *et al*., 2016; Yamamoto *et al*., 2016). The MED64 assay was conducted according to a previously reported method (Asakura *et al*., 2015). The criteria for the assay were as follows: amplitude of the first peaks of the field potential waveform (sodium amplitude) ≥ ± 200 μV, amplitude of the second peaks of the field potential waveform ≥ 15 μV, number of first peaks per minute (beating rate) > 34 beats/min and field potential duration (FPD) > 340 msec (Asakura *et al*., 2015).

The hiPS-CMs were seeded at 2.5-3.0 × 10^4 cells/2 μL/probe to the MED probe (MED-PG515A, Alpha Med Scientific) and cultured in a 5% CO_2 incubator (37°C, with humidification). Approximately three hours later, 1 mL of culture medium was added to each MED probe, and the cells were cultured. Thereafter, the culture medium was replaced with fresh medium every other day. After seven days, the cell sheets were used in the assay. The culture medium was replaced with fresh medium at the 2 mL/probe and the cells were cultured in a 5% CO_2 incubator for more than 1 hr to overnight. The MED probe with the cell sheet was set in the MED64 device (Alpha Med Scientific), and stabilized for more than 30 min at 37°C, in 5% CO_2. The waveform of field potential of the spontaneously beating cell sheets was recorded through a 0.1 Hz high-pass filter and a 5 kHz low-pass filter for 10 min before and 10 min after applying 2 μL of each concentration of the drugs (in 6 individual preparations).

The data analyses

The FDSS/μCELL assay

The data were presented as the 50% peak width from the peak head of Ca fluorescence waveform (PWD), which was used as a surrogate of QT (Hayakawa *et al*., 2014). The concentrations of the drugs that prolonged the PWD by ≥ 10% in comparison to vehicle treatment (PWD10), and the number of peak heads of the Ca fluorescence waveforms per minute (P-rate) was counted automatically. These parameters were presented as the mean value of four individual preparations.

The Ca fluorescence waveforms corresponding to the electrocardiogram waveform and the cardiac action potential waveform are illustrated in Fig. 1. The 90% peak width of the Ca fluorescence waveform is thought to be a better QT surrogate; however, it could not be calculated because it was too close to the baseline of Ca fluorescence when the baseline showed a high degree of fluctuation.

The MED64 assay

The data were presented as the field potential duration (FPD), a surrogate of the QT (Asakura *et al*., 2015; Hayakawa *et al*., 2014), which was calculated from the last 30 beats at each concentration. The FPD was corrected using the correction formula *of Bazett* and *Fridericia* (FPDcB and FPDcF, respectively). The concentrations that prolonged the FPD, FPDcB and FPDcF by ≥ 10%, were shown as FPD10, FPDcB10 and FPDcF10, respectively. The beating rate was calculated for the last 30 beats of the field potential waveform. The occurrence of early after-depolarization (EAD) as surrogates of arrhythmias (Asakura *et al*., 2015) and arrest, were observed during the field potential recording period.

The field potential waveforms corresponding to the electrocardiogram waveform, action potential waveform, and EAD waveform are illustrated in Fig. 1.

RESULTS

The FDSS/μCELL assay

1) E-4031 and cisapride: Reported to prolong QT in both humans and animals

The PWD10 of E-4031 (0.003, 0.01, 0.03, 0.1, 0.3 μmol/L) was 0.03 μmol/L (Fig. 2, Table 1), and the P-rate decreased along with the increase in the drug concentration (≥ 0.03 μmol/L) (Fig. 2). The PWD10 of cisapride (0.01, 0.03, 0.1, 0.3, 1 μmol/L) was ≤ 0.01 μmol/L (Fig. 2, Table 1), and the P-rate decreased at concentrations of ≥ 0.03 μmol/L (Fig. 2).

2) E-8010, diphenhydramine and famotidine: Reported to prolong QT in humans but not always in animals

The PWD10 of E-8010 (0.01, 0.03, 0.1, 0.3, 1 μmol/L) was 0.03 μmol/L (Fig. 2, Table 1), and the P-rate was not dramatically changed at concentrations of up to 1 μmol/L (Fig. 2). Diphenhydramine (1, 3, 10, 30, 100 μmol/L)

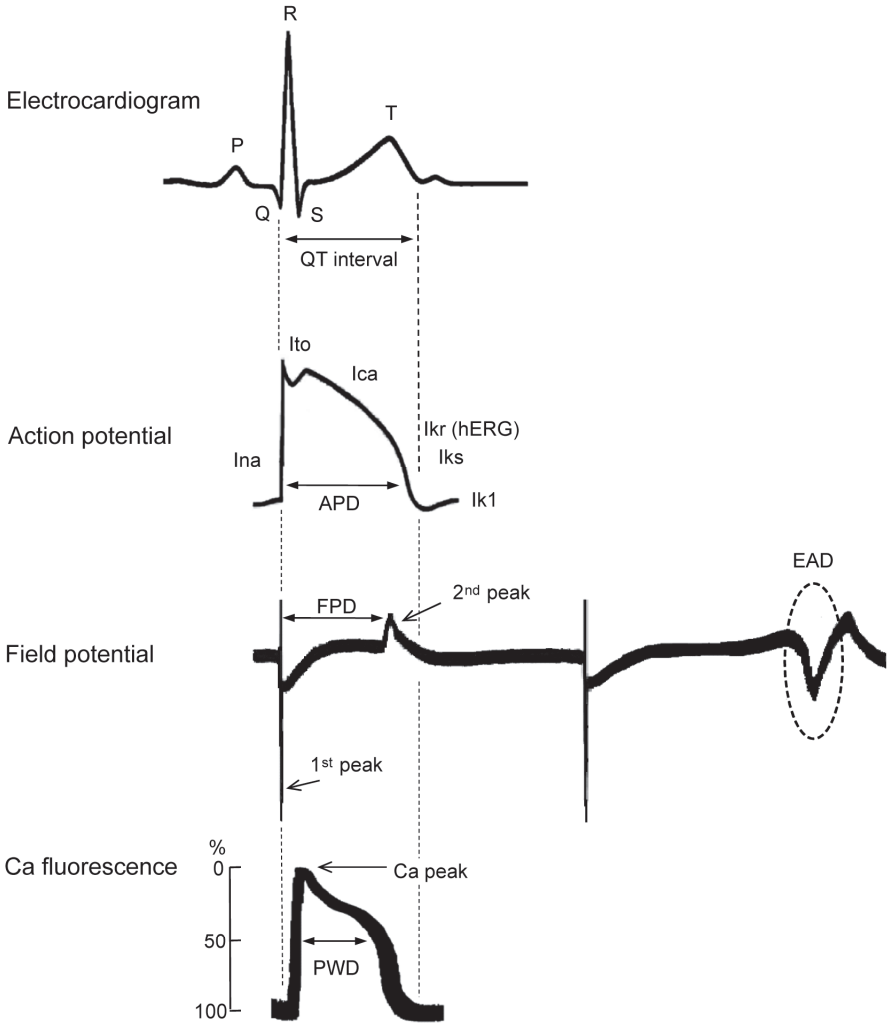

Fig. 1. A schematic representation of the Ca fluorescence waveform in the FDSS/μCELL system, the field potential waveform in the MED64 system and the action potential waveform measured using the whole-cell current clamp technique, which corresponds to the electrocardiogram trace in humans. The action potential-related ion channel current is shown in the action potential waveform drawing (above). APD: action potential duration, FPD: field potential duration, EAD: early after-depolarization, PWD: calcium fluorescence peak width, Ina: sodium ion current, Ito: early outward potassium current, Ica: calcium ion current, Ikr: cardiac rapidly activating delayed rectifier potassium current, hERG: human ether-à-go-go-related gene, Iks: cardiac slowly-activating delayed rectifier potassium current, Ik1: main inward rectifier potassium current, Ca: calcium.

prolonged PWD in 1/4 preparation at a concentration of 30 μmol/L and caused arrest in 3/4 and 4/4 preparations at concentrations of 30 μmol/L and 100 μmol/L, respectively (Fig. 2, Table 1). The P-rate was not affected at concentrations of up to 10 μmol/L (Fig. 2). Famotidine (10, 30, 100 μmol/L) prolonged the PWD at concentrations of ≥ 30 μmol/L (Fig. 2, Table 1) and the P-rate was increased slightly at concentrations of ≥ 30 μmol/L (Fig. 2).

3) Nifedipine and verapamil: Reported to have no effect on the QT in humans but to sometimes affect the QT in animals

Nifedipine (0.01, 0.03, 0.1, 0.3, 1 μmol/L) shortened the PWD at concentrations of ≥ 0.03 μmol/L (Fig. 2, Table 1), and the P-rate increased as the drug concentration increased (≥ 0.03 μmol/L) (Fig. 2). Verapamil (0.01, 0.03, 0.1, 0.3, 1 μmol/L) shortened the PWD at concentrations of ≥ 0.3 mol/L (Fig. 2, Table 1), and the P-rate increased as the drug

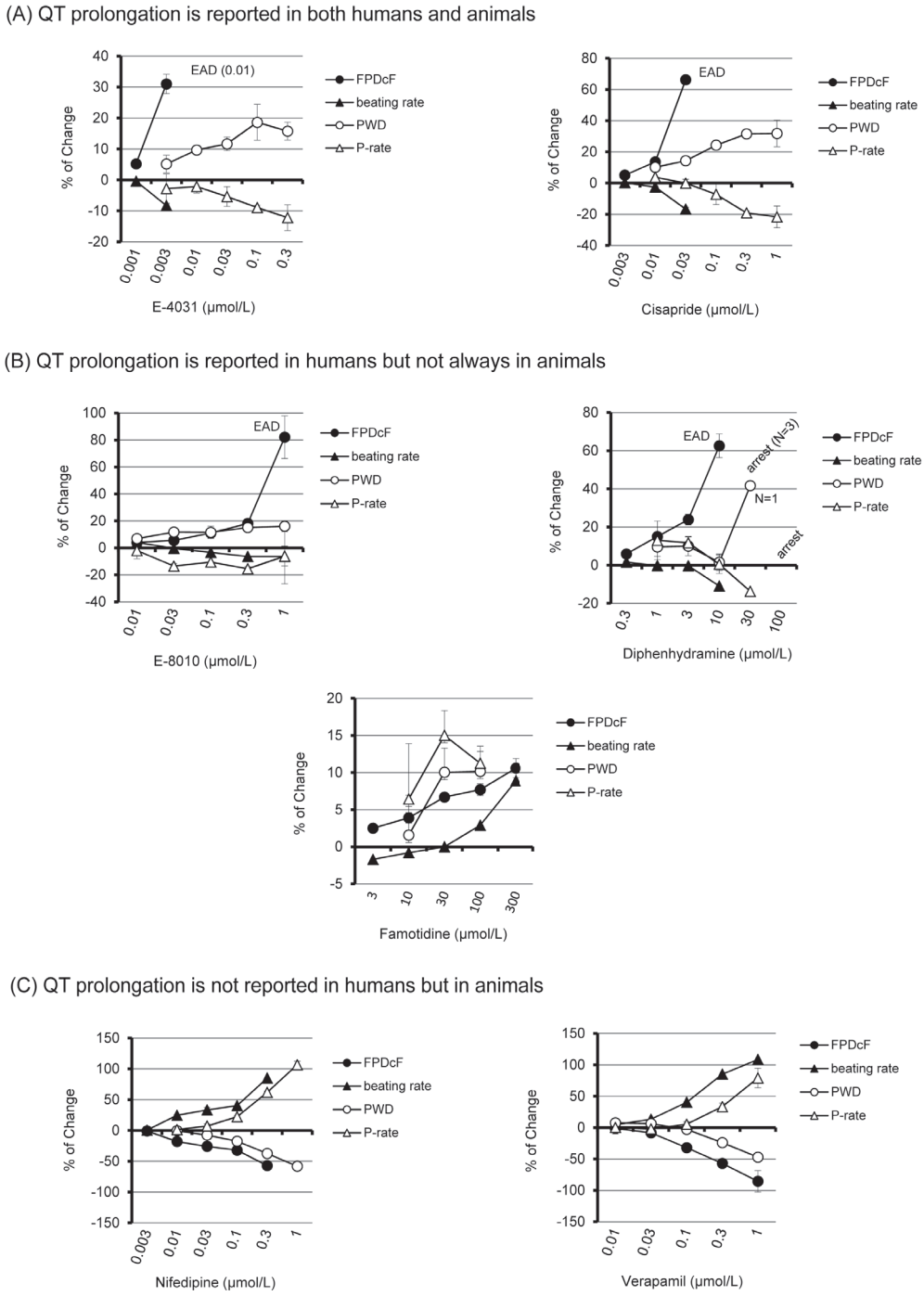

Fig. 2. Effects of drugs on the cardiac functional parameters of cardiomyocytes derived from human iPS cells. (A) Effects of E-4031 and cisapride, which are reported to prolong the QT in both humans and animals, (B) effects of E-8010, diphenhydramine and famotidine, which are reported to prolong the QT in humans but not always in animals, (C) effects of nifedipine and verapamil, which are reported to have no effect on the QT in humans but to affect the QT in animals only sometimes. Each point with a bar represents the mean ± S.E. of four or six preparations. The open symbols represent the FDSS/μCELL assay, and the closed symbols represent the MED64 assay. PWD: calcium fluorescence peak width, P-rate: beating rate (beats/min). FPDcF: field potential duration corrected with *Fridericia's* formula, EAD: early after-depolarization, beating rate: beats/min.

concentration increased (≥ 0.3 μmol/L) (Fig. 2).

The MED64 assay

1) E-4031 and cisapride: Reported to prolong the QT in both humans and animals

E-4031 (0.001, 0.003, 0.01, 0.03 μmol/L) prolonged the FPD; the FPD10, FPDcB10 and FPDcF10 were all 0.003 μmol/L and caused EAD at a concentration of 0.01 μmol/L (Fig. 2, Table 1). E-4031 reduced the beating rate at concentrations of ≥ 0.003 μmol/L (Fig. 2). Cisapride (0.003, 0.01, 0.03, 0.1 μmol/L) prolonged the FPD, and the FPD10, FPDcB10 and FPDcF10 were all 0.01 μmol/L and caused EAD at a concentration of 0.03 μmol/L (Fig. 2, Table 1). Cisapride decreased the beating rate at concentrations of ≥ 0.03 μmol/L (Fig. 2). These results were in accordance with the results of previous studies (Kitaguchi *et al*., 2016; Nozaki *et al*., 2016; Yamamoto *et al*., 2016).

2) E-8010, diphenhydramine and famotidine: Reported to prolong the QT in humans but not always in animals

E-8010 (0.01, 0.03, 0.1, 0.3, 1 μmol/L) prolonged the FPD, and the FPD10, FPDcB10 and FPDcF10 were all 0.1 μmol/L (Fig. 2, Table 1). E-8010 caused EAD at 1 μmol/L and the beating rate was not increased at concentrations of up to 0.3 μmol/L (Fig. 2, Table 1). Diphenhydramine (0.3, 1, 3, 10, 30 μmol/L) prolonged the FPD, and the FPD10, FPcB10 and FPDcF10 were all 1 μmol/L (Fig. 2, Table 1). Diphenhydramine caused EAD at 10 μmol/L, and the beating rate was not affected at concentrations of up to 3 μmol/L (Fig. 2, Table 1). Famotidine (3, 10, 30, 100, 300 μmol/L) prolonged the FPD at a concentration of 300 μmol/L, and the FPDcB10 and FPDcF10 were 300 μmol/L; the FPD10 was not prolonged (Fig. 2, Table 1). The beating rate was increased slightly at concentrations of ≥ 100 μmol/L (Fig. 2). Famotidine did not cause EAD at concentrations of up to 300 μmol/L (Fig. 2, Table 1).

3) Nifedipine and verapamil: Reported to have no effect on QT in humans but to sometimes affect the QT in animals

Nifedipine (0.003, 0.01, 0.03, 0.1 μmol/L) shortened the FPD along with the increase in concentration (≥ 0.01 μmol/L) and did not cause EAD (Fig. 2, Table 1). The beating rate increased with increasing concentrations (≥ 0.01 μmol/L) (Fig. 2). Verapamil (0.01, 0.03, 0.1, 0.3, 1 μmol/L) shortened the FPD with increasing concentrations (≥ 0.03 μmol/L) and did not cause EAD (Fig. 2, Table 1). The beating rate increased with increasing concentrations (≥ 0.03 μmol/L) (Fig. 2).

DISCUSSION

E-4031, a class III antiarrhythmic drug, is a representative hERG/Ikr inhibitor that prolongs the QT by inhibiting the Ikr/hERG channel in humans, dogs and monkeys (Ando *et al*., 2005; Vargas *et al*., 2015). In the MED64 assay, E-4031 prolonged the FPD, a QT surrogate, and led to the occurrence of EAD, an arrhythmia surrogate, at the same nanomolar concentration that inhibited the hERG/Ikr channel. The concentrations that caused FPD10 (0.003 μmol/L) and EAD (0.01 μmol/L) were the same concentrations as that were reported in a previously published study (Kitaguchi *et al*., 2016). In the FDSS/μCELL assay E-4031 prolonged the PWD but did not cause EAD. This assay used a half-size plate when it was first time performed; thus, the experimental conditions, such as the cell density, might not have been optimal, which might have been why EAD was undetected. In another FDSS/μCELL assay, EAD was caused by E-4031 in a co-culture of iCell® cardiomyocytes[2] and human cardiac fibroblasts at a volume ratio (3:1) that was equivalent to that of the human heart (Camelliti *et al*., 2005). It was suggested that the QT prolongation and *torsade de pointes* caused by E-4031 in the clinical setting could be predicted using hiPS-CMs. This paper does not discuss the protein binding of drugs. Because high protein binding would be associated with low clearance and low protein binding would be associated with high clearance in the *in vivo* setting and because cardiotoxicity depends on the unbound drug concentration in cardiac tissue, the author is of the opinion that the unbound drug concentration calculated from protein binding using human or animal plasma protein would not always correspond with the unbound concentration in human cardiac tissue. Furthermore, the experimental medium contains serum protein.

Cisapride, a prokinetic drug, is a 5-HT_3 antagonist and a 5-HT_4 agonist and a well-known hERG/Ikr inhibitor. This drug prolongs the QT in humans, dogs and monkeys by inhibiting the hERG/Ikr channel (Ando *et al*., 2005; Vargas *et al*., 2005). This drug prolonged the PWD and FPD at the same nanomolar concentration that caused hERG/Ikr channel inhibition in the present study; the concentrations that caused FDP10 (0.01 μmol/L) and EAD (0.03 μmol/L) in the MED64 assay were almost the same as those reported in a previous study (Nozaki *et al*., 2016). These findings suggest that the clinical QT prolongation and *torsade de pointes* caused by cisapride could be predicted using hiPS-CMs.

E-8010, a drug used to treat erectile dysfunction, is a

Table 1. Summary of the effects of the drugs on the calcium fluorescence peak width and field potential duration of cardiomyocytes derived from human iPS cells.

Drug	Concentration (μmol/L)	FDSS/μCELL	MEA				hERG/Ikr	QT prolongation			Therapeutic plasma concentration (μmol/L)[1,6,12-14]
		PWD10 (μmol/L)	FPD10 (μmol/L)	FPDcB10 (μmol/L)	FPDcF10 (μmol/L)	EAD (μmol/L)	IC_{50} (μmol/L)[1-4]	Monkey (μmol/L)[1,5,6]	Dog (μmol/L)[1,7-9]	Human (μmol/L)[1,3,10,11]	
QT is prolonged in both humans and animals											
E-4031 (Ikr antagonist)	0.001*, 0.003, 0.01, 0.03, 0.1#, 0.3#	+ 0.03		+ 0.003		+ 0.01	+ 0.008-0.02	+ 0.0872	+ 0.020	+ Tdp: 0.011-0.033	0.0035-0.013
Cisapride ($5HT_3$ antagonist/ $5HT_4$ agonist)	0.003*, 0.01, 0.03, 0.1, 0.3#, 1#	+ ≤ 0.01		+ 0.01		+ 0.03	+ 0.014-0.027	+ 0.260	+ 0.307	+ Tdp: 0.176-0.301	0.120-0.170
QT is prolonged in humans but not always in animals											
E-8010 (PDE5 inhibitor)	0.01, 0.03, 0.1, 0.3, 1	+ 0.03		+ 0.1		+ 1	+ 0.0407	- (1000 mg/kg, p.o.)	+ 0.984 ± 0.078[a]	+ (≥ 20 mg, p.o.)	0.183
Diphenhydramine (H_1 antagonist)	0.3*, 1, 3, 10, 30, 100#	+ 30 ≥ 30 (arrest)		+ 1		+ 10	+ 2.6-30	- (10 mg/kg, p.o.)	- (10 mg/kg, p.o.)	+ Tdp (Over dose, ≥ 500mg, p.o.)	0.086-0.171 (50 mg, p.o.: 0.233-0.287)
Famotidine (H_2 antagonist)	3* 10, 30, 100, 300*	+ 30	-	+ 300		-	+ > 100	ND	- (10 mg/kg, i.v.)[b]	+ Tdp (20 mg, i.v., bid)	0.05-0.11
QT is not prolonged in humans, but not always in animals											
Nifedipine (Ca antagonist)	0.003*, 0.01, 0.03, 0.1, 0.3, 1#	- Shortening ≥ 0.03		- Shortening ≥ 0.01		-	+ 275	ND	+ (0.3 mg/kg, p.o.)	-	0.035-0.194
Verapammil (Ca antagonist)	0.01, 0.03, 0.1, 0.3, 1	- Shortening ≥ 0.3		- Shortening ≥ 0.03		-	+ 0.136-0.8	+ (5 mg/kg, p.o.) or - (15 mg/kg, p.o.)	+ (5 mg/kg, p.o.)	-	0.025-0.081

PWD10: concentration of 10% prolongation of 50% peak width, FPD10: concentration of 10% prolongation of field potential duration, FPDcB10: concentration of 10% prolongation of field potential duration corrected with *Buzett's* formula, FPDcF10: concentration of 10% prolongation of field potential duration corrected with *Fridericia's* formula, EAD: concentration of incidence of early after-depolarization, MEA: multielectrode array, +: prolongation/positive, -: no-effect/negative, Tdp: occurence of *torsades de pointes*, ND: no data, Ikr: rapidly activating potassium channel, Iks: slowly activating potassium channel, $5HT_3$: serotonin-3 receptor, $5HT_4$: serotonin-5 receptor, PDE5: phosphodiesterase type-5, H_1: histamine-1 receptor, H_2: histamine-2 receptor, #: FDSS/CELL assay only tested, *: MED64 assay only tested. a) 10 μg/kg/mL infusion, b) In human, therapeutic dose is 0.3 mg/kg (i.v.).

1) Adachi *et al*., 2014; 2) Kirsch *et al*., 2004; 3) Omata *et al*., 2005; 4) Yun *et al*., 2015; 5) Ando *et al*., 2005; 6) Kitaguchi *et al*., 2016; 7) Vargas *et al*., 2015; 8) Volders *et al*., 2003; 9) Sugiyama *et al*., 2003; 10) Tekbaş *et al*., 2012; 11) Lee *et al*., 2004; 12) Gul *et al*., 2013; 13) Echizen *et al.*, 1988; 14) Mohammad *et al*., 1997.

phosphodiesterase type-5 inhibitor that prolongs the QT in humans but not in monkeys (Adachi *et al*., 2014). This drug has no effect on the cardiovascular system of monkeys at concentrations of up to 1000 mg/kg (p.o.), but QT prolongation was triggered in phase 1 clinical trials at doses of ≥ 20 mg/person, and the clinical development was terminated. Thereafter it was confirmed that it caused QT prolongation in anesthetized dogs and hERG/Ikr inhibition (Mizuno *et al*., 2003). In the present study, E-8010 prolonged the PWD and FPD by ≥ 10% at concentrations less than the plasma concentration (0.183 μmol/L) in clinical therapy; at a higher concentration (1 μmol/L), EAD occurred (in the MEA assay only). E-8010 inhibits the hERG current at a concentration of the nanomolar order (IC_{50} = 0.040 μmol/L) (Mizuno *et al*., 2003). These findings suggested that E-8010 prolongs the PWD and FPD at a concentration of the same order as that which inhibits the hERG current. Thus, the assay using hiPS-CMs could predict not only clinical QT prolongation but also the risk of *torsade de pointes* due to E-8010.

Diphenhydramine, a histamine H_1-receptor antagonist, one of the most commonly used drugs, is available over-the-counter. This drug prolongs the QT in humans, but not in dogs or monkeys (Ando *et al*., 2005; Vargas *et al*., 2015). In the MEA assay this drug prolonged the FPD at a concentration that was higher than that used in clinical therapy (0.233-0.287 μmol/L); it also caused EAD at concentration that was higher than that used in clinical therapy. On the other hand, in the FDSS/μCELL assay, diphenhydramine caused arrest or PWD prolongation at concentrations that were higher than the therapeutic concentration. These diphenhydramine-induced effects occurred at a concentration that was similar to that which caused hERG/Ikr current inhibition (IC_{50} = 2.6-30 μmol/L) (Kirsch *et al*., 2004; Omata *et al*., 2005). These results suggest that an overdose of diphenhydramine would cause QT prolongation or *torsades de pointes* in humans. Actually, diphenhydramine-induced QT prolongation in humans was caused by a dose that was approximately 10 times the therapeutic dose (Zareba *et al*., 1997; Husain *et al*., 2010).

Famotidine, an anti-ulcer drug, is a histamine H_2-receptor antagonist that prolongs the QT in humans but not in dogs (Sugiyama *et al*., 2003; Vargas *et al*., 2015). In the present study, the PWD and the FPD were prolonged at higher than therapeutic concentrations. This drug does not inhibit the Ikr/hERG current at therapeutic concentrations, but inhibits it at higher concentrations (> 100 μmol/L) (Yun *et al*., 2015). These findings suggested that the PWD and FPD prolongations caused by famotidine would occur due to hERG/Ikr inhibition. QT prolongation very rarely occurs in association with famotidine. The mechanisms through which famotidine causes QT prolongation have not been fully clarified; however, famotidine would prolong the QT by inhibiting the hERG/Ikr current in patients with increasing drug sensitivity owing to the cardiovascular risk factors (*i.e.,* hypertension, diabetes, and dyslipidemia), patients with other therapeutic agents (drug interaction), and patients with renal impairment (lead to higher plasma drug concentrations). On the other hand, the H_2-receptors are located in the human heart, modulate the cardiac function (Eckel *et al*., 1982; McNeill, 1984; Baller and Huchzermeyer, 1989; Kim *et al*., 2006; Francis and Wilson Tang, 2006). Famotidine has negative effects on the cardiac function in humans (Kirch *et al*., 1992). Another H_2-receptor antagonist, ranitidine, prolongs the QT by inhibiting cholinesterase at the nerve endings (Tekbas *et al*., 2012). The famotidine-induced QT prolongation may be caused by the direct or indirect effects on the patient's cardiomyocytes.

Nifedipine, an anti-hypertensive drug, is a dihydropyridine L-type Ca channel antagonist. It has no effect on the QT in humans, but prolongs the QT in dogs (Ando *et al*., 2005; Vargas *et al*., 2015). In the present study, nifedipine shortened the PWD and FPD at therapeutic concentrations. The inhibition of the cardiac Ca channel reduces the entry of Ca^{2+} into the cardiomyocytes, and the second phase (plateau phase) of the action potential waveform is decreased (shortened until the repolarization time), and the duration of the action potential is shortened. It is suggested that nifedipine would shorten the PWD and the FPD by inhibiting the cardiac Ca channel. Thus, it seems likely that nifedipine cannot cause QT prolongation by acting directly on cardiomyocytes. QT shortening would therefore not be induced in clinical therapy because nifedipine acts more markedly on the peripheral vasculature than on the heart.

Verapamil, an anti-hypertension drug, is a phenylalkylamine L-type Ca channel antagonist. It has no effect on the QT in humans but prolongs the QT in dogs and monkeys (Ando *et al*., 2005; Vargas *et al*., 2015). In the present study, verapamil shortened the PWD and FPD at higher than therapeutic concentrations. The mechanisms underlying the shortening of PWD and FPD shortening were suggested to be the same those of nifedipine: the action potential duration and repolarization time were shortened by the inhibition of the cardiac Ca channel. Verapamil inhibited the hERG/Ikr current at the tested concentrations; however, the PWD and FPD were not prolonged. Some drugs that cause hERG/Ikr inhibition, such as anti-cancer drugs, do not prolong the QT in humans. These findings for verapamil are one example of

a case in which hERG/Ikr current inhibition does not necessarily cause QT prolongation. Verapamil is not expected to cause QT shortening in clinical therapy because it acts more on the sinus node than on cardiomyocytes and thereby decreases the heart rate.

The findings of the present study suggest that the hiPS-CMs-based assay would be a useful tool for predicting the clinical risk of cardiotoxicity that directly affects the cardiomyocytes. The MED64 assay was considered to be highly sensitive in predicting the QT prolongation and proarrhythmias in humans. The FDSS/μCELL assay is a high-throughput test. The optimal experimental conditions, such as the optimal density for cell seeding, the optimal carbon dioxide concentration in the measurement environment and other factors should be determined for the half-size volume plate.

ACKNOWLEDGMENTS

The author is grateful to Mr. Takuji Kataoka at Hamamatsu Photonics for conducting the measurement and performing the analysis using the FDSS/μCELL system.

Conflict of interest---- The author declares that there is no conflict of interest.

REFERENCES

Adachi, H., Geary, E.S. and Tsukidate, K. (2014): Safety pharmacology assessment for a drug development candidate dropped due to QT prolongation during phase 1 clinical development. In Tanimoto Gatsukou Dokusei Shitsumombako, pp.83-90, Scientist Press, Tokyo, Japan.

Ando, K., Hombo, T., Kanno, A., Ikeda, H., Imaizumi, M., Shimizu, N., Sakamoto, K., Kitani, S., Yamamoto, Y., Hizume, S., Nakai, K., Kitayama, T. and Yamamoto, K. (2005): QT product: *in vivo* QT assay with a conscious monkey for assessment of the potential for drug-induced QT interval prolongation. J. Pharmacol. Sci., **99**, 487-500.

Asakura, K., Hayashi, S., Ojima, A., Taniguchi, T., Miyamoto, N., Nakamori, C., Nagasawa, C., Kitamura, T., Osada, T., Honda, Y., Kasai, C., Ando, H., Kanda, Y., Sekino, Y. and Sawda, K. (2015): Improvement of acquisition and analysis methods in multi-electrode array experiments with iPS cell-derived cardiomyocytes. J. Pharmacol. Toxicol. Meth., **75**, 17-26.

Baller, D. and Huchzermeyer, H. (1989): Histamine effects on the heart with special reference to cardiac side effects of H_2 receptor antagonists. Klin. Wochenschr., **67**, 743-755.

Camelliti, P., Borg, T.K. and Kohl, P. (2005): Structure and functional characterization of cardiac fibroblasts. Cardiovasc. Res., **65**, 40-51.

Eckel, L., Gristwood, R.W., Hawrath, H. and Owen, D.A.A. (1982): Inotropic and electrophysiological effects of histamine on human ventricular heart muscle. J. Physiol., **330**, 111-123.

Echizen, H., Shoda, R., Umeda, N. and Ishizaki, T. (1988): Plasma famotidine concentration versus intragastric pH in patients with upper gastrointestinal bleeding and in healthy subjects. Cli. Pharmacol. Ther., **44**, 690-698.

Francis, G.S. and Wilson Tang, W.H. (2006): Histamine, mast cells, and heart failure. J. Am. Coll. Cardiol., **48**, 1385-1386.

Gul, L., Coyle, L., Abrams, R.M.-C., Kemper, R., Chiao, E.T. and Kolaja, K.L. (2013): Refining the human iPS-cardiomyocyte arrhythmic risk assessment model. Toxicol. Sci., **136**, 581-594.

Hay, M., Thomas, D.W., Craighead, J.L., Economides, C. and Rosenthal, J. (2014): Clinical development success rates for investigation drugs. Nat. Biotech., **32**, 40-51.

Hayakawa, T., Kunihiro, T., Ando, T., Kobayashi, S., Matsui, E., Yada, H., Kanda, Y., Kurosaka, J. and Furukawa, T. (2014): Image-based evaluation of contraction-relaxation kinetics of human-induced pluripotent stem cell-derived cardiomyocytes: correlation and complementarity with extracellular electrophysiology. J. Mol. Cell. Cardiol., **77**, 178-191.

Husain, Z., Hussain, K., Nair, R. and Steinman, R. (2010): Diphenhyrdamine induced QT prolongation and torsade de pointes: an uncommon effect of a common drug. Cardiol. Res., **17**, 509-511.

Kim, J., Ogai, A., Nakatani, S., Hashimura, K., Kanzaki, H., Komamura, K., Asakura, M., Asanuma, H., Kitamura, S., Tomoike, H. and Kitakaze, M. (2006): Impact of blockade of histamine H_2 receptors on chronic heart failure revealed by retrospective and prospective randomized studies. J. Am. College Cardiol., **48**, 1378-1384.

Kirch, W., Hakabi, A. and Hinrichsen, H. (1992): Hemodynamic effects of quinidine and famotidine in patients with congestive heart failure. Clin. Pharmacol. Ther., **51**, 325-333.

Kirsch, G.E., Trepakova, E.S., Brimecombe, J.C., Sidach, S.S., Erickson, H.D., Kochan, M.C., Shyjka, L.M., Lacerda, A.E. and Brown, A.M. (2004): Variability in the measurment of hERG potassium channel inhibition: effects of temperature and stimulus pattern. J. Pharmacol. Toxicol. Meth., **50**, 93-101.

Kitaguchi, T., Moriyama, Y., Taniguchi, T., Ojima, A., Ando, H., Uda, T., Otabe, K., Oguchi, M., Shimizu, S., Saito, H., Morita, M., Toratani, A., Asayama, M., Yamamoto, W., Matsumoto, E., Saji, D., Ohnaka, H., Tanaka, K., Washio, I. and Miyamoto, N. (2016): CSAHi study: Evaluation of multi-electrode array in combination with human iPS cell-derived cardiomyocytes to predict drug-induced QT prolongation and arrhythmia-effect of 7 reference compounds at 10 facilities. J. Pharmacol. Toxicol. Meth., **78**, 93-102.

Lee, K.W., Kayser, S.R., Hongo, R.H., Tseng, Z.H. and Scheinman, M.M. (2004): Famotidine and long QT syndrome. Am. J. Cardiol., **93**, 1325-1327.

Lu, Z., Kamiya, K., Opthof, T., Yasui, K. and Kodama, I. (2001): Density and kinetics of Ikr and Iks in guinea pig and rabbit ventricular myocytes explain different efficacy of Iks implications for arrhythmogenesis in human. Circulation, **104**, 951-956.

McNeill, J.H. (1984): Histamine and heart. Can. J. Physiol. Pharmacol., **62**, 720-726.

Mizuno, H., Adachi, H., Kimura, J., Sawa, Y., Kakiki, M., Lansdell, K., Saito, M. and Kerns, W.D. (2003): Cardiovascular assessment of ER-118585, a selective phosphodiesterase 5 inhibitor. Biol. Pharm. Bull., **26**, 1661-1667.

Mohammad, S., Zhou, Z., Gonng, Q. and January, C.T. (1997): Blockade of the HERG human cardiac K+ channel by the gastrointestinal prokinetics agent cisapride. Am. J. Pharmacol., **273**, 2538-2538.

Nozaki, Y., Honda, Y., Watanabe, H., Saiki, S., Koyabu, K., Itoh,

T., Nagasawa, C., Nakamori, C., Nakayama, C., Iwasaki, H., Suzuki, S., Washio, I., Takahashi, E., Miyamoto, K., Yamanishi, A., Endo, H., Shinozaki, J., Nogawa, H. and Kunimatsu, T. (2016): CSAHi study: validation of multi-electrode array systems (MEA60/2100) for prediction of drug-induced proarrhythmia using human iPS cell-derived cardiomyocytes-assessment of inter-facility and cells lot-to-lot-variability. Regul. Toxicol. Pharmacol., **77**, 75-86.

O'hara, T. and Rudy, Y. (2012): Quantitative comparison of cardiac ventricular myocyte electrophysiology and response to drugs in human and nonhuman species. Am. J. Physiol. Heart Circ. Physiol., **302**, H1023-H1030.

Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., Dorato, M., Deun, K.V., Smith, P., Berger, B. and Heller, A. (2000): Concordance of the toxicity of pharmaceuticals in humans and animals. Regul. Toxicol. Pharmacol., **32**, 56-67.

Omata, T., Kasai, C., Hashimoto, M., Hombo, T. and Yamamoto, K. (2005): QT product: Comparison of non-clinical studies for drug-induced delay in ventricular repolarization and their role in safety evaluation in human. J. Pharmacol Sci., **99**, 531-541.

Peng, S., Lacerda, A.E., Kirsch, G.E., Brown, A.M. and Bruening-Wright, A. (2010): The action potential and comparative pharmacology of stem cell-derived human cardiomyocytes. J. Pharmacol. Toxicol. Meth., **61**, 277-286.

Redfern, W.S., Carlsson, L., Davis, A.S., Lynch, W.G., MacKenzie, I., Palethorpe, S., Siegl, P.K.S., Strang, I., Sullivan, A.T., Wallis, R., Camm, A.J. and Hammond, T.G. (2003): Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovasc. Res., **58**, 32-45.

Sugiyama, A., Satoh, Y., Takahara, A., Nakamura, Y., Shimizu-Sasamata, M., Sato, S., Miyata, K. and Hashimoto, K. (2003): Famotidine does not induce long QT syndrome: experimenal evidence from in vitro and in vivo test systems. Eur. J. Pharmacol., **466**, 137-146.

Tamaki, C., Nagayama, T., Hashiba, M., Fujiyoshi, M., Hizue, M., Kodaira, H., Nishida, M., Suzuki, K., Takashima, Y., Ogino, Y., Yasugi, D., Yoneta, Y., Hisada, S., Ohkura, T. and Nakamura, K. (2013): Potentials and limitations of nonclinical safety assessment for predicting clinical adverse drug reactions: correlation analysis of 142 approved drugs in Japan. J. Toxicol. Sci., **38**, 581-598.

Tekbaş, E., Arıtürk, Z., Cil, H. and İslamoğlu, Y. (2012): Famotidine-induced acquired long QT syndrome: a case report. Gaziantep Tip Derg., **18**, 48-50.

Vargas, H.M., Bass, A.S., Koerner, J., Matis-Mitchell, S., Pugsley, M.K., Skinner, M., Burnham, M., Bridgland-Taylor, M., Pettit, S. and Valentin, J.P. (2015): Evaluation of drug-induced QT interval prolongation in animal and human studies: a literature review of concordance. Bri. J. Pharmacol., **172**, 4002-4011.

Voldera, P.G.A., Stengl, M., van Opstal, J.M., Gerlach, U., Spatjens, R.L.H.M.G., Beekman, J.D.M., Sipido, K.R. and Vos, M.A. (2003): Probing the contribution of Iks to canine ventricular repolarization. Circulation, **107**, 2753-2760.

Watkins, P.B. (2011): Drug safety sciences and the bottleneck in drug development. Clin. Pharmacol. Ther., **89**, 788-790.

Yamamoto, W., Asakura, K., Hiroyuki, A., Ando, H., Taniguchi, T., Ojima, A., Uda, T., Osada, T., Hayashi, S., Kasai, C., Miyamoto, N., Tashibu, H., Yoshinaga, T., Yamazaki, D., Sugiyama, A., Kanda, Y., Sawada, K. and Sekino, Y. (2016): Electrophysiological characteristics of human iPS-derived cardiomyocytes for the assessment of drug-induced proarrhythmic potential. PLoS One, **11**, e0167348.

Yun, J., Hwangbo, E., Lee, J., Chon, C.R., Kim, P.A., Jeong, I.H., Park, M., Park, R., Kang, S.J. and Choi, D. (2015): Analysis od an ECG record database reveals QT interval prolongation potential of famotidine in a large korean polution. Cardiovasc. Toxicol., **15**, 197-202.

Zareba, W., Mass, A.J., Rosero, S.Z., Hajj-Ali, R., Konecki, J. and Andrews, M. (1997): Electrocardiographic findings in patients with diphenhydramine overdose. Am. J. Cardiol., **80**, 1168-1173.