Inhibitory effects of class I antiarrhythmic agents on Na\(^+\) and Ca\(^{2+}\) currents of human iPS cell-derived cardiomyocytes

Sayaka Yonemizu\(^a,1\), Keiichiro Masuda\(^a,1\), Yasutaka Kurata\(^b,\ast\), Tomomi Notsu\(^a\), Yuhei Higashi\(^a\), Kenta Fukumura\(^a\), Peili Li\(^a\), Haruaki Ninomiya\(^c\), Junichiro Mika\(^d\), Motokazu Tsuneto\(^a\), Yasuaki Shirayoshi\(^a\), Ichiro Hisatome\(^a\)

\(^a\) Division of Regenerative Medicine and Therapeutics, Department of Genetic Medicine and Regenerative Therapeutics, Tottori University Graduate School of Medical Science, 86 Nishi-cho, Yonago 683-8503, Japan
\(^b\) Department of Physiology II, Kanazawa Medical University, 1-1 Daigaku, Uchinada-machi, Kahoku-gun, Ishikawa 920-0293, Japan
\(^c\) Department of Biological Regulation, Tottori University Faculty of Medicine, Yonago 683-8503, Japan
\(^d\) Department of Pharmacology, Tottori University Faculty of Medicine, Yonago 683-8503, Japan

Abstract

Introduction: Human induced pluripotent stem cells (hiPSCs) harboring cardiac myosin heavy chain 6 promoter can differentiate into functional cardiomyocytes called “iCell cardiomyocytes” under blastis- cindin treatment condition. While iCell cardiomyocytes are expected to be used for predicting cardio- toxicity of drugs, their responses to antiarrhythmic agents remain to be elucidated. We first examined electrophysiological properties of iCell cardiomyocytes and mRNA levels of ion channels and Ca handling proteins, and then evaluated effects of class I antiarrhythmic agents on their Na\(^+\) and Ca\(^{2+}\) currents.

Methods: iCell cardiomyocytes were cultured for 8–14 days (38–44 days after inducing their differentia- tion), according to the manufacturer’s protocol. We determined their action potentials (APs) and sarcolemmal ionic currents using whole-cell patch clamp techniques, and also mRNA levels of ion channels and Ca handling proteins by RT-PCR. Effects of three class I antiarrhythmic agents, pirmenol, pilsicainide and mexiletine, on Na\(^+\) channel current, Na\(^+\) channel current (INa), L-type Ca\(^{2+}\) channel current (ICaL) and slowly-activating delayed-rectifier K\(^+\) channel current were measured.

Results: iCell cardiomyocytes revealed sinoatrial node-type (18%), atrial-type (18%) and ventricular-type (64%) spontaneous APs. The maximum peak amplitudes of INa, ICaL, and Na\(^+\) channel current were 62.7 \pm 13.7, –8.1 \pm 0.7, and 3.0 \pm 1.0 pA/pF, respectively. The hyperpolarization-activated cation channel and inward-rectifier K\(^+\) channel currents were observed, whereas the T-type Ca\(^{2+}\) channel or slowly-activating delayed-rectifier K\(^+\) channel current was not detectable. mRNAs of Nav1.5, Cav1.2, Kv1.4, KvLQT1, hERG and SERCA2 were detected, while that of HCN1, minK or MiRP was not. The class I antiarrhythmic agent pirmenol and class Ib agent pilsicainide blocked INa in a concentration-dependent manner with IC50 of 0.87 \pm 0.37 and 0.88 \pm 0.16 \mu M, respectively; the class Ib agent mexiletine revealed weak INa block with a higher IC50 of 30.0 \pm 0.37 m\mu M. Pirmenol, pilsicainide and mexiletine blocked ICaL with IC50 of 2.00 \pm 0.39, 7.7 \pm 2.5 and 5.0 \pm 0.1 \mu M, respectively.

Conclusions: In iCell cardiomyocytes, Icao was blocked by the class Ia and Ic antiarrhythmic agents and ICaL was blocked by all the class I agents within the ranges of clinical concentrations, suggesting their cardiotoxicity.

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Original Article
1. Introduction

Cardiomyocytes derived from the human induced pluripotent stem cell (hiPSC) as well as the human embryonic stem cell provide new in vitro platforms for drug discovery and evaluation of cardiac toxicities of compounds. iCell cardiomyocytes are well known to be hiPSC-derived cardiomyocytes harboring blasticidin resistance gene driven by the cardiac myosin heavy chain 6 gene (MYH6) promoter; in the presence of blasticidin, therefore, iCell cardiomyocytes expressing MYH6 are selectively purified as human ventricular myocytes [1]. Thus, the iCell cardiomyocyte is an appropriate model for evaluating adverse effects of antiarrhythmic agents on human ventricular myocytes. However, electrophysiological properties of iCell cardiomyocytes, their expressions of ion channel and Ca handling protein mRNAs, and effects of therapeutic drugs, especially class I antiarrhythmic agents, on their electrophysiological properties remain to be elucidated.

Class I antiarrhythmic agents, blocking Na⁺ channels, are worldwide used for patients with supraventricular and ventricular arrhythmias [2]. However, several clinical trials such as Cardiac Arrhythmia Suppression Trial (CAST-I) demonstrated that class Ic antiarrhythmic agents significantly increased the mortality of patients with myocardial infarction [3,4]. Although mechanisms responsible for adverse effects of class Ic antiarrhythmic agents remain unknown, their negative inotropic actions may trigger proarrhythmic events by negatively affecting hemodynamic conditions of the patients [5,6]. To predict cardiac toxicity of class I antiarrhythmic agents, heterologous cell models expressing ion channels of the human heart or animal models are frequently used in the preclinical stage; however, the differences in cell types or species hinder the accurate prediction of their cardiotoxicity in human cases.

In the present study, we confirmed the electrophysiological properties of iCell cardiomyocytes as well as their expressions of mRNAs, and examined blocking actions of class I antiarrhythmic agents on the Na⁺ channel current (INa) and L-type Ca²⁺ channel current (ICaL) in the iCell cardiomyocyte.

2. Materials and methods

2.1. Cell culture

iCell cardiomyocytes [Cellular Dynamics International (CDI), Madison, WI, USA; Lot#: CMC021544, 1290129] were cryopreserved and stored in liquid nitrogen; they were grown for 30 days by the manufacturer CDI before being shipped. For this study, single vials containing 1.5 × 10⁶ cardiomyocytes were thawed by immersing the frozen cryovial in a 37 °C water bath; thawed cardiomyocytes were transferred into a 15-ml tube, and diluted with 10 ml of ice-cold plating medium [iCell Cardiomyocytes Plating Medium (iCPM); CDI]. iCell cardiomyocytes can be maintained in culture for 14 days using the Maintenance Medium (CDI) without appreciable loss of purity, according to the manufacturer’s manual. iCell cardiomyocytes were cultured for another 8–14 days, i.e., cultured for 38–44 days after inducing differentiation, until they were subjected to patch-clamp experiments and RT-PCR analysis.

2.2. Electrophysiology

iCell cardiomyocytes (4 × 10⁴ to 8 × 10⁴ cells/well) were cultured on cover slips coated with fibronectin and were subjected to patch clamp experiments. The action potential (AP) and sarcolemmal ion channel currents were measured by the whole-cell patch-clamp technique with an Axopatch-200 B amplifier (Molecular Devices, San Jose, CA, USA) as described elsewhere [7]. Generation of command voltage pulses, data acquisition and data analyses were performed with the pCLAMP 9 software (Molecular Devices). Cells were perfused with normal Tyrode’s solution of the following composition (mM): 140 NaCl, 5.4 KCl, 1.8 CaCl₂, 0.5 MgCl₂, 0.33 NaHPO₄, 5 HEPES, and 5 glucose (pH 7.4 with NaOH). The internal pipette solution contained (in mM) 130 K-glutamate, 1 MgCl₂, 15 KCl, 5 NaOH, 5 HEPES, and 5 Mg-ATP (pH 7.3 with KOH). Recording pipettes had tip resistances of <5 MΩ when filled with the internal solution. Cell membrane capacitances were 410 ± 4.8 pF (mean ± SEM). INa was elicited at 0.2 Hz by 300-ms depolarization pulses of −80 to −40 mV (in 10 mV increments) from a HP of −90 mV; ICaL and outward K⁺ currents were eliminated by 5 μM Cd²⁺ and 5 μM E4031 which almost completely block ICaL and the rapid component of delayed-rectifier K⁺ channel currents (IKr), respectively. lkal was elicited at 0.2 Hz by 300-ms depolarization pulses of −40 to +40 mV (in 10 mV increments) from a HP of −50 mV. IKr and the slow (IKs) components of the delayed-rectifier K⁺ channel current were elicited every 5 s by 2-s depolarization pulses of −40 to +40 mV (in 10 mV increments) from a HP of −50 mV. The hyperpolarization-activated cation channel current (Ih) was elicited every 10 s by 3-s hyperpolarizations of −80 to −150 mV (in 10 mV decrements) from a HP of −50 mV. In addition, the inward-rectifier K⁺ channel current (IK1) was recorded using a ramp pulse protocol with a function generator (FG-122, NF Corporation, Yokohama, Japan); the ramp pulses from −120 to 0 mV with a HP of −40 mV were applied at 1.5 V/s every 3 s. Most of experiments were carried out at 37 °C, while INa was measured at 27 °C.

To determine the blocking effects of class I antiarrhythmic agents on Na⁺ and L-type Ca²⁺ channels of iCell cardiomyocytes, INa and ICaL were measured during a train of 20-ms step depolarizations to −20 and 0 mV, respectively, at 0.2 Hz in the presence of the agents; the amplitudes of peak INa and ICaL reached steady-state levels at the 10th pulse, their steady-state values determined at the 20th pulse. Concentration dependence of the effects of each drug on peak INa and ICaL was fitted using the following equation:

\[ I% = \frac{(100−R%) \times IC_{50}^B}{IC_{50}^B + [D]^h} + R% \]  

where I% represents the percentages of peak INa and ICaL determined at −20 mV and 0 mV, respectively, in the presence of a drug at a given concentration ([D]), with those in the absence of the drug being set as 100%. R% denotes the residual (non-blocked) current (%) at higher concentrations. IC₅₀ and h are the half-maximal inhibitory concentration and Hill coefficient, respectively. All data are presented as the mean ± SEM.

2.3. RT-PCR assay

Total RNAs of iCell cardiomyocytes were isolated using an RNAeasy Micro Kit (QiAGEN, Tokyo, Japan). CDNAs were synthesized using PrimeScript® RT reagent Kit with gDNA Eraser Perfect Real Time (Takara Bio, Otsu, Japan). RT-PCR was performed using EmeraldAmp® MAXPCR Master Mix (Takara). Primer sequences are listed in Table 1.

3. Results

3.1. Electrical properties of APs in iCell cardiomyocytes

iCell cardiomyocytes revealed automaticity of sinoatrial node (SAN)-type, atrial-type and ventricular-type APs, which are classified according to the criteria of Ma et al. [1] (Fig. 1). SAN-type iCell
cardiomyocytes showed clearer phase 4 depolarization than atrial-type cells. Ventricular-type APs had longer duration and the prominent plateau phase. Out of 22 cells, SAN-, atrial- and ventricular-type APs were observed in 4 (18%), 4 (18%) and 14 (64%) cells, respectively. The SAN-type APs were characterized by the maximum diastolic potential (MDP) of $-52.3 \pm 2.7$ mV, over shoot potential (OS) of $29.8 \pm 5.2$ mV, AP amplitude (APA) of $72.0 \pm 5.4$ mV, AP duration (APD) at 50% repolarization (APD50) of $110 \pm 50$ ms, APD at 50% repolarization (APD90) of $260 \pm 20$ ms, and AP interval of $470 \pm 50$ ms. The atrial-type APs were characterized by the MDP of $-57.2 \pm 3.3$ mV, OS of $26.6 \pm 1.0$ mV, APA of $73.7 \pm 4.1$ mV, APD50 of $130 \pm 10$ ms, APD90 of $290 \pm 30$ ms, and AP interval of $490 \pm 60$ ms. The ventricular-type APs were characterized by the MDP of $-61.1 \pm 3.0$ mV, OS of $37.7 \pm 5.3$ mV, APA of $88.8 \pm 7.0$ mV, APD50 of $200 \pm 40$ ms, APD90 of $340 \pm 50$ ms, and AP interval of $640 \pm 50$ ms.

### 3.2. Characteristics of sarcolemmal ionic currents in iCell cardiomyocytes

We next tried recording sarcolemmal ionic currents, $I_{Na}$, $I_{CaL}$, T-type Ca$^{2+}$ channel current ($I_{CaT}$), $I_{Ks}$, $I_{K1}$, $I_{f}$ and $I_{K1}$, in iCell cardiomyocytes. Fig. 2A shows the original traces of $I_{Na}$ elicited by 300-ms depolarizing pulses from a HP of $-90$ mV. Peak current-voltage relationship of $I_{Na}$ revealed the threshold potential of $-60$ mV and the maximum peak current of $-62.7 \pm 13.7$ pA/pF at $-30$ mV (n = 10). Fig. 2B shows the original traces of $I_{CaL}$ elicited by 300-ms depolarization pulses from a HP of $-90$ mV. Peak current-voltage relationship revealed the threshold potential of $-40$ mV and the maximum peak current of $-8.1 \pm 0.7$ pA/pF at $-10$ mV (n = 10). We also tried to detect $I_{CaT}$ using more negative HP, but could not demonstrate its presence. $I_{K1}$ was determined as the E4031-sensitive current, i.e., the difference between currents recorded in the absence and presence of 1 μM E4031, during 2-s depolarizing pulses from a HP of $-50$ mV and repolarization pulses to the HP (Fig. 2C); E4031 at 1 μM abolished tail currents during the step repolarization, isolating the E4031-sensitive $I_{K1}$. Current-voltage relationship of $I_{K1}$ determined at the end of depolarization pulses revealed the threshold potential of $-30$ mV and the maximum current amplitude of $3.0 \pm 1.0$ pA/pF at $+30$ mV. The voltage-dependence of $I_{K1}$ peak tail currents showed that $I_{K1}$ activated at the potentials positive to $-40$ mV to reach maximum at $+10$ mV, with the maximum peak tail currents of $0.57 \pm 0.06$ pA/pF. On the other hand, the chromanol 293 B-sensitive $I_{K1}$ was not observed in iCell cardiomyocytes (Supplemental Fig. S1A); chromanol 293 B did not prolong their APD (Supplemental Fig. S1B). Fig. 2D shows original traces of membrane currents elicited by 3-s hyperpolarizing pulses from a HP of $-50$ mV in the absence and presence of 2 mM Cs⁺ by which $I_{f}$ is almost completely blocked. A threshold potential of the Cs⁺-sensitive $I_{f}$ was $-80$ mV and the maximum currents of $3.2 \pm 0.8$ pA/pF were evoked at $-140$ mV. Membrane current recording by ramp pulses with a HP of $-40$ mV demonstrated the presence of $I_{K1}$ with the slope conductance of $102 \pm 10$ pS/pF (n = 10), which was almost completely blocked by Ba²⁺ at $3$ mM (Supplemental Fig. S2).

### 3.3. mRNA expression profile of iCell cardiomyocytes

Fig. 3 shows mRNA levels of ion channels and Ca handling proteins in iCell cardiomyocytes on 44 days after differentiation. mRNAs of the ion channels Nav1.5, Cav1.2, Kir2.1, HCN4, KvLQT1 and hERG as well as the myocin light chain 2v (Mlc2v) and 2a (Mlc2a) were detected, while that of HCN1, mink or MiRP was not. With respect to Ca handling proteins, mRNAs of RyR2 and SERCA2 were not, but not that of the inositol 1,4,5-triphosphate receptor type 2 (IP3R2), were detected.

### 3.4. Concentration-dependent inhibitory effects of class I antiarrhythmic agents on $I_{Na}$ and $I_{CaL}$ in iCell cardiomyocytes

Next, we studied the concentration-dependent effects of class I antiarrhythmic agents, pirmenol (class Ia), mexiletine (class Ib) and pilsicainide (class Ic), on $I_{Na}$ and $I_{CaL}$. Fig. 4 shows the original traces of $I_{Na}$ recorded at $-20$ mV in the absence and presence of these agents. Pirmenol and pilsicainide at $1$ μM each remarkably blocked $I_{Na}$, while mexiletine at the same concentration of $1$ μM did not. Concentration dependence curves obtained from multiple experiments (n = 7–11) indicated that pirmenol, mexiletine and
pilsicainide blocked INa with IC50 values of 0.87 ± 0.37, 30.0 ± 3.0, and 0.88 ± 0.16 μM, respectively, and the Hill coefficients of 0.91 ± 0.32, 0.84 ± 0.56 and 1.30 ± 0.24, respectively. Fig. 5 shows the original traces of ICaL recorded at 0 mV in the absence and presence of these agents. Pirmenol and pilsicainide at 1 μM each significantly blocked ICaL, while mexiletine at the same concentration only slightly blocked ICaL. Concentration dependence curves obtained from multiple experiments (n = 7–11) showed that pirmenol, mexiletine and pilsicainide blocked ICaL with IC50 of 2.0 ± 0.4, 5.0 ± 0.1, and 7.7 ± 2.5 μM, respectively, and the Hill coefficient of 1.27 ± 0.25, 1.28 ± 0.10 and 0.62 ± 0.07, respectively.

4. Discussion

In the present study, we confirmed the electrophysiological properties and mRNA expressions of iCell cardiomyocytes, and then examined the effects of three class I antiarrhythmic agents on INa and ICaL.

4.1. Electrophysiological properties and mRNA expression of iCell cardiomyocytes

We classified spontaneous APs of iCell cardiomyocytes into three different types of ventricular-, SAN- and atrial-type APs, based on the following criteria of Ma et al. [1]: Ventricular-type APs have larger amplitude with hyperpolarized MDP and longer APD with distinct plateau phase, while SAN- and atrial-type APs have smaller amplitude with minimal plateau phase. SAN-type APs show more depolarized MDP with more distinct phase 4 depolarization than atrial-type APs. In this study, the percentages of the ventricular-, SAN-, and atrial-type APs were 64%, 18%, and 18%, respectively.
respective, which are very close to those in the previous report of Ma et al. [1] (58%, 22% and 24%, respectively). Taken together, iCell cardiomyocytes showed predominantly ventricular-type APs, but revealed the heterogeneous characteristics including various types of cardiac phenotypes even when cells were purified using MYH6 reporter assays, as reported previously [1].

The largest and the second-largest currents in iCell cardiomyocytes recorded in the present study were I_Na and I_CaL, respectively; the maximum peak current densities of I_Na and I_CaL averaged −62.7 and −8.1 pA/pF, respectively, which were smaller than those in the previous reports for adult human atrial and ventricular myocytes [8,9] as well as hiPSC-derived cardiomyocytes [1]. The APD values of ventricular-type APs were smaller in this study than in the previous reports, probably reflecting the smaller I_CaL. However, the threshold and peak potentials of both currents were comparable to those in the previous studies. In the present study, we used iCell cardiomyocytes on 38–44 days after differentiation. According to a previous report [10], mRNA levels of Nav1.5 and Cav1.2 in hiPSC-derived cardiomyocytes on 45 days after differentiation were comparable to those in human adult heart tissues, suggesting that the functions of Na⁺ and Ca²⁺ channels (densities of I_Na and I_CaL) in the hiPSC-derived cardiomyocyte may be close to those in human adult heart tissues. Thus, the iCell cardiomyocyte could be useful for evaluating the toxicity of Na⁺ and Ca²⁺ channel blockers to human hearts, e.g., their effects on the resting potential and phase-3 repolarization of ventricular myocytes, the effects of I_Ks inhibitors on APs may be less prominent in iCell cardiomyocytes than in native human ventricular myocytes. The mRNA levels of Kir2.1 in iCell cardiomyocytes on 45 days after differentiation was much less than that in human adult heart tissues [10]. Thus, in iCell cardiomyocytes on 38–44 days after differentiation, the expression of Kir2.1 would be less than that in human adult heart tissues, which is consistent with the present electrophysiological data of the reduced expression of Kir2.1 mRNA.

It is of interest that iCell cardiomyocytes expressed I_f channel, which is known to be encoded mainly by the HCN gene family, the marker for SAN cells [20]. Previous studies using hiPSC-derived cardiomyocytes indicated that they expressed I_f channels encoded by HCN1, 2 and 4. It was reported that in human ES cell-derived cardiomyocytes on 57 days after differentiation, HCN1 mRNA decreased without changes in HCN4, and the activation of I_f slowed, suggesting that both the reduction of HCN1 and slowed activation of I_f may reflect maturation of hiPSC-derived cardiac myocytes as well [21]. In the present study, iCell cardiomyocytes on 38–44 days after differentiation did not express HCN1 mRNA but expressed HCN2 and 4, suggesting that iCell cardiomyocytes reach a steady state of maturation on 38 days or later. We could not tell maturation from the aspect of I_f activation kinetics, since we could not precisely measure the rate of I_f activation at the early stage of iCell cardiomyocytes.

Taken together, the electrophysiological properties of iCell cardiomyocytes determined in the present study were quantitatively different in AP waveforms and channel current densities from those of human ventricular myocytes in the previous reports, but were qualitatively the same as in the previous reports. These results suggest that iCell cardiomyocytes could be useful for evaluating the cardiotoxicity of antiarrhythmic drugs, although their AP responses to the agents may vary depending on lots (clones).

The maturity of iCell cardiomyocytes has been evaluated by measurements of gene expression, ion channel functions, intracellular Ca²⁺ cycling, responsiveness to cardioactive pharmacological stimuli and metabolism [10]; on 45 days after differentiation, mRNA levels of Nav1.5 and Cav1.2 in iCell cardiomyocytes reached as high as those in the adult heart tissue, associated with increases of I_Na and I_CaL densities as well as
increased intracellular Ca\textsuperscript{2+} concentrations and mRNA level of SERCA2. Responsiveness to endothelin 1 (ET-1) and insulin-like growth factor-1 (IGF-1) can be used as a benchmark for measuring mature degrees of signaling pathways in hiPSC-derived cardiomyocytes relative to those in isolated mature human cardiac myocytes. iCell cardiomyocytes on 45 days after differentiation responded to both ET-1 and IGF-1, indicating that the myocytes acquire a functionally mature phenotype by 45 days after differentiation [10]. It has also been reported that in cardiac myocytes derived from the human ES cell (H1), mRNAs of HCN1 and HCN4 were expressed at the late stage (day 57–110) than at the early stage (day 16–25), while mRNAs of Kir2.1, Kv4.3 and Kv1.4 increased at the late stage [21]. iCell cardiomyocytes did not express HCN1 but expressed Nav1.5, Cav1.2, Kir2.1, SERCA2 and Mlc2v, suggesting that their maturation level is comparable to that at the late stage of hiPSC-derived cardiomyocytes.

4.2. Effects of class I antiarrhythmic agents on \( I_{\text{Na}} \) of iCell cardiomyocytes

Class I antiarrhythmic agents block \( I_{\text{Na}} \) to suppress supraventricular and ventricular arrhythmias. They are categorized into 3 types of class Ia, Ib and Ic based on the Vaughan Williams classification, i.e., from the aspect of their actions on APDs of ventricular myocytes [22]. As shown in Table 2, the class Ia antiarrhythmic agent pirmenol [23] and class Ic agent pilscainide [24] are used to treat supraventricular and ventricular arrhythmias at clinical blood concentrations of 5.23 \( \pm \) 0.59 and 2.08 \( \pm \) 0.53 \( \mu \text{M} \), respectively. The class Ib antiarrhythmic agent mexiletine [25] is used for the treatment of ventricular arrhythmias at a clinical concentration of 6.08 \( \pm \) 1.12 \( \mu \text{M} \). Qu et al. [26] reported that human ES cell-derived cardiomyocytes were less sensitive to these Na\textsuperscript{+} channel blockers. Gibson et al. [27] showed that a class Ic antiarrhythmic agent, flecainide, slowed AP upstroke in iCell cardiomyocytes, but did not examine the direct action of the agent on \( I_{\text{Na}} \). This is the first report to show the inhibitory actions of class I antiarrhythmic agents on \( I_{\text{Na}} \) in iCell cardiomyocytes. In the present study, the IC\textsubscript{50} values of \( I_{\text{Na}} \) block by pirmenol and pilscainide were lower than their clinical blood concentrations in patients who have been prescribed with pirmenol or pilscainide for ventricular arrhythmias, suggesting that the class Ia and Ic antiarrhythmic agents sufficiently block \( I_{\text{Na}} \) of human ventricular myocytes in the clinical setting. On the other hand, the IC\textsubscript{50} value of mexiletine-induced \( I_{\text{Na}} \) block (30 \( \mu \text{M} \) on average) was much larger than its clinical concentrations. In the present study, the steady-state amplitude of peak \( I_{\text{Na}} \) in the presence of the agent was measured at the 20th pulse during a train of the 20-ms step depolarizations at 0.2 Hz. Different potencies of the agents for \( I_{\text{Na}} \) block could be due to differences in kinetics of the binding to and unbinding from Na\textsuperscript{+} channels [28]: Since pirmenol and pilscainide belong to the slow kinetic drug [29], blocked Na\textsuperscript{+} channels are accumulated even when depolarization pulses are applied at the low frequency of 0.2 Hz as in the present study, suggesting that they sufficiently block \( I_{\text{Na}} \) at the clinical concentrations. In contrast, mexiletine belongs to the fast kinetic drug [29] so that blocked Na\textsuperscript{+} channels are not accumulated during the low frequency depolarizing pulses. Mexiletine would not significantly block \( I_{\text{Na}} \) of the human ventricular myocyte at a clinical concentration, although it could block \( I_{\text{Na}} \) under tachycardia or in a state of membrane depolarization.

The inhibitory actions of class I antiarrhythmic agents on \( I_{\text{Na}} \) have been evaluated using cultured cells transfected with cDNA of human Nav1.5 \( \alpha \) subunit. As shown in Table 2, IC\textsubscript{50} values of \( I_{\text{Na}} \) block by mexiletine [30] and pilscainide [31] obtained using transfected cells were much higher than their clinical concentrations, suggesting that co-expression of \( \alpha \) and \( \beta \) subunits is necessary for the agents to block Na\textsuperscript{+} channels on the plasma membrane potently. Thus, iCell cardiomyocytes could be useful for the evaluation of actions of class I antiarrhythmic agents on human ventricular Na\textsuperscript{+} channels, while not suitable for evaluating the action of KS inhibitors because of the lack of minK expression and resulting very small \( I_{\text{Ks}} \).

\( I_{\text{Na}} \) channel blockers are well known to decrease the intracellular Na\textsuperscript{+} activity in ventricular myocytes and thereby cause the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger-mediated reduction of the intracellular Ca\textsuperscript{2+} concentration which leads to negative inotropic effects [32]. Thus, the inhibition of \( I_{\text{Na}} \) by pirmenol and pilscainide at clinical concentrations, stronger than that by mexiletine, may impair the cardiac contractile function through decreases in the intracellular Na\textsuperscript{+} activity.

4.3. Effects of class I antiarrhythmic agents on \( I_{\text{CaL}} \) of iCell cardiomyocytes

It is also well known that the amount of Ca\textsuperscript{2+} releases from the sarcoplasmic reticulum depends on the sarcolemmal Ca\textsuperscript{2+} influx through L-type Ca\textsuperscript{2+} channels [33]. Therefore, the degree of \( I_{\text{CaL}} \) block is the primary determinant of the difference in negative inotropic effects among antiarrhythmic agents. In the present study, the class I antiarrhythmic agents also blocked \( I_{\text{CaL}} \) with different IC\textsubscript{50} and Hill coefficient values, suggesting the binding of the class I agents to L-type Ca\textsuperscript{2+} channels with different kinetics. iCell cardiomyocytes may also be useful for evaluation of blocking actions of class I agents on human ventricular L-type Ca\textsuperscript{2+} channels. As shown in Table 2, the IC\textsubscript{50} values of \( I_{\text{CaL}} \) inhibitions by pirmenol, mexiletine and pilscainide in iCell cardiomyocytes were close to their clinical concentrations (2.08–6.08 \( \mu \text{M} \)); the class I antiarrhythmic agents may impair the cardiac contraction by significantly blocking \( I_{\text{CaL}} \) in the clinical setting. Taken together, the combined inhibition of \( I_{\text{Na}} \) and \( I_{\text{CaL}} \) may explain an adverse effect, impaired contractility, of class I antiarrhythmic agents on human ventricular myocytes.

4.4. Limitation of study

There are several limitations of this study. In the present study, we used only two lots for iCell cardiomyocytes. Therefore, the

| Class | IC\textsubscript{50} values (\( \mu \text{M} \)) | Expression systems | Clinical concentrations (C\textsubscript{max}) | MW (g/mol) |
|-------|-----------------------------|----------------|-----------------------------|-----------|
| pirmenol | 0.87 \( \pm \) 0.37 | Nav 1.5 block | 1770 \( \pm \) 200 | 338.4864 |
| mexiletine | 30 \( \pm \) 3.0 | Nav 1.5 block | 1090 \( \pm \) 200 | 179.2588 |
| pilscainide | 0.88 \( \pm \) 0.16 | Nav 1.5 block | 566.8 \( \pm \) 145.2 | 272.3853 |

ND: not determined.

\( ^a \) Determined at a holding potential of \(-120 \text{ mV} \) [Ref. [30]].

\( ^b \) Determined at a holding potential of \(-90 \text{ mV} \) [Ref. [31]].
effects of the class Ib agent mexiletine on INa is relatively weak with significance for the expressions of some proteins such as minK and MiRP. This study has diversity of their AP waveforms is attributable to impaired ex-K channels by class-I antiarrhythmic drugs studied by using the maestro MEA platform. Evidence for the possible involvement of Ca2+ entry blockade in the relaxation by class I antiarrhythmic drugs in the isolated pig coronary smooth muscle. J Mol Cell Cardiol 1987;19:367.

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5. Conclusion

We examined electrophysiological properties, mRNA expression of ion channels as well as Ca handling proteins, and responses to class I antiarrhythmic agents of the iCell cardiomyocyte that is the hiPSC-derived cardiac myocyte. We found that the mRNA expression profile of Nav1.5, Cav1.2, Kir2.1 and HCN1 was similar to that in human adult ventricular tissues, although iCell cardiomyocytes showed a diversity of AP waveforms, i.e., ventricular, SAN- and atrial-type APs, and expressed INa, ICaL, Ik, and IKr but not ICaT or ICa. It is the subject in a future study to determine whether the AP waveforms is attributable to impaired expressions of some proteins such as mink and MirP. This study has for the first time demonstrated that INa in iCell cardiomyocytes is significantly blocked by the class la antiarrhythmic agent pirmenol and class lc agent pilcainside at clinical concentrations, while the effect of the class lb agent methidrine on INa is relatively weak with the IC50 higher than clinical concentrations. Ical in iCell cardiomyocytes was significantly blocked by all the class I agents in the range of their clinical concentrations.

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Appendix A. Supplementary data

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