Toxicology and Applied Pharmacology 296 (2016) 42–53

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Evaluation of nefazodone-induced cardiotoxicity in human induced pluripotent stem cell-derived cardiomyocytes

Sujeong Lee [a,1], Hyang-Ae Lee [a,b,c,1], Sung Woo Choi [b], Sung Joon Kim [b,*,2], Ki-Suk Kim [a,c,**,2]

[a] Next-generation Pharmaceutical Research Center, Korea Institute of Toxicology, Korea Research Institute of Chemical Technology, 141 Gajeong-ro, Yuseong-gu, Daejeon 305-343, South Korea
[b] Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, 110-799, South Korea
[c] Human and Environmental Toxicology Program, University of Science and Technology, 217 Gajeong-ro, Yuseong-gu, Daejeon 305-350, South Korea

ARTICLE INFO

Article history:
Received 6 May 2015
Revised 12 January 2016
Accepted 21 January 2016
Available online 25 January 2016

Keywords:
Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs)
Cardiotoxicity
Nefazodone
Patch clamp
Action potentials
Ion channels

ABSTRACT

The recent establishment of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), which express the major cardiac ion channels and recapitulate spontaneous mechanical and electrical activities, may provide a possible solution for the lack of in vitro human-based cardiotoxicity testing models. Cardiotoxicity induced by the antidepressant nefazodone was previously revealed to cause an acquired QT prolongation by hERG channel blockade. To elucidate the cellular mechanisms underlying the cardiotoxicity of nefazodone beyond hERG, its effects on cardiac action potentials (APs) and ion channels were investigated using hiPSC-CMs with whole-cell patch clamp techniques. In a proof of principle study, we examined the effects of cardioactive channel blockers on the electrophysiological profile of hiPSC-CMs in advance of the evaluation of nefazodone. Nefazodone dose-dependently prolonged the AP duration at 90% (APD_{90}) and 50% (APD_{50}) repolarization, reduced the maximum upstroke velocity (dV/dt_{max}) and induced early after depolarizations. Voltage-clamp studies of hiPSC-CMs revealed that nefazodone inhibited various voltage-gated ion channel currents including I_{Kr}, I_{Ks}, I_{Na}, and I_{Ca}. Among them, I_{Kr} and I_{Na} showed relatively higher sensitivity to nefazodone, consistent with the changes in the AP parameters. In summary, hiPSC-CMs enabled an integrated approach to evaluate the complex interactions of nefazodone with cardiac ion channels. These results suggest that hiPSC-CMs can be an effective model for detecting drug-induced arrhythmogenicity beyond the current standard assay of heterologously expressed hERG K^+ channels.

1. Introduction

Several types of in vitro and in vivo QT screening systems are currently in use with standardized protocols. However, none of the assay systems reflects the genuine properties of the human cardiac cells yet. The recently established human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) can be used as an in vitro preclinical model to predict cardiotoxicity. Unlike primary culture models of human cardiomyocytes, which lose their ability to beat in the process of proliferation, hiPSC-CMs express cardiac contractile proteins and functional ionic channels to allow the physical contraction of myocytes (Anson et al., 2011; Ma et al., 2011, Cohen et al., 2011, Guo et al., 2011a, 2011b). The development of an in vitro system based on hiPSC-CMs could provide more relevant human cell lines for drug safety assessment in a reproducible manner (Davis et al., 2012, Pugsley et al., 2011, Redfern and Valentin, 2011). Although the endogenous electrophysiological properties of hiPSC-CMs have been well reported (Peng et al., 2010, Ma et al., 2011), there is a paucity of data concerning drug-induced responses that validate hiPSC-CMs as an in vitro preclinical model for toxicological evaluation.

Antidepressants for depressive disorders also affect the autonomic nerves innervating the heart and are associated with cardiovascular mortality (Nicholson et al., 2006; Roose and Miyazaki, 2005, Jiang and Davidson, 2005, Whooley et al., 2008). Although a second-generation antidepressant, nefazodone (Owens et al., 1995, Schatzberg, 2000, Eison et al., 1990), has been initially claimed to have significantly less cardiovascular side effects than the first-generation tricyclic antidepressants (Miura et al., 2014, DeVane et al., 2002; Greene and Barbhaiya, 1997), an increasing number of studies on nefazodone have raised concerns regarding its cardiac safety. Such concerns were raised by a case of

* Correspondence to: S.J. Kim, Department of Biomedical Sciences, College of Medicine, Seoul National University, 103 Daehak-ro, Jongro-gu, Seoul 110-799, South Korea.
** Correspondence to: K.S. Kim, Department of Next-generation Pharmaceutical Research Center, Korea Institute of Toxicology, Korea Research Institute of Chemical Technology, 141 Gajeong-ro, Yuseong-gu, Daejeon 305-343, South Korea.
E-mail addresses: crystalee@gmail.com (S. Lee), hyangaelee@gmail.com (H.-A. Lee), djmaya@snu.ac.kr (S.W. Choi), sjoonkim@snu.ac.kr (S.J. Kim), idkks00@gmail.com (K.-S. Kim).
[1] These authors (S Lee and HA Lee) contributed equally to this work as first authors.
[2] These authors (SJ Kim and KS Kim) contributed equally to this work as corresponding authors.

http://dx.doi.org/10.1016/j.taap.2016.01.015
0041-008X/

QT prolongation and torsade de pointes (TdP) tachyarrhythmia that occurred in a patient receiving nefazodone (Isbister and Hackett, 2003, Rasimas and Burkhart, 2006, Fernandez et al., 2007, Siddiqui and Khan, 2004). We have recently found that nefazodone inhibits human ether-a-go-go related gene (hERG, KCNH2 or Kv11.1) channels by interacting with the aromatic binding sites Y652 and F656 within the S6 domain of hERG (Shin et al., 2014). Although the likely mechanism behind the drug-induced prolongation of the QT interval is the interaction of nefazodone with hERG channels (Sanguinetti and Tristani-Firouzi, 2006, Katchman et al., 2006), not all hERG blockers cause QT prolongation or TdP, and additional genes associated with the QT interval can cause life-threatening cardiac arrhythmias (Goldenberg and Moss, 2008). In addition, mutations in genes that encode cardiac ionic channels, such as KCNQ1/KCNE1-encoded slow component of the delayed rectifier potassium current (I_{Ks}) channels, KCNH2-encoded I_{Kr} channels, and SCN5A-encoded inward voltage-gated sodium current (I_{Na}) channels, can disrupt the fine balance among ionic currents and lead to life-threatening arrhythmias (Curran et al., 1995, Wang et al., 1995, 1996).

A new paradigm for cardiac arrhythmic safety assessment in a non-clinical in vitro human model relies upon the analysis of effects of drugs on the action potentials (APs) and various types of cardiac ion currents. Thus, to adopt hiPSC-CMs for the assay system, it is necessary to verify the relevance to the pharmacological/toxicological effects on the ionic currents in heterologous expression systems. To understand the mechanisms of arrhythmogenicity of nefazodone, we investigate the effects of various conventional cardiac ion channels blockers as well as nefazodone on hiPSC-CMs and HEK293 cells expressing cardiac ion channels. To elucidate the usefulness of hiPSC-CMs in in vitro preclinical toxicological evaluation, we examined whether (1) hiPSC-CMs recapitulate appropriate electrical response of cardioactive selective channel blockers; (2) hiPSC-CMs could be used to evaluate cardiotoxicity of nefazodone, previously known as a non-cardiac acting drug.

2. Materials and methods

2.1. Cell culture

The hiPSC-CMs (iCell Cardiomyocytes; Cellular Dynamics International, Madison, WI, USA) were cultured for single-cell electrophysiological recordings. Frozen vials of hiPSC-CMs were thawed in a water bath maintained at 37 °C and mixed with ice-cold plating medium (iCell Cardiomyocyte Plating Medium). The cells were transferred to four-well culture plates containing 0.1% gelatin-coated glass coverslips at a low density to yield single uncoupled cells. Then, the cells maintained in a culture incubator at 37 °C in an atmosphere of 93% air and 7% CO_2. After 2 days of culture, the plating medium was replaced with culture medium (iCell Cardiomyocyte Maintenance Medium), which was then changed every 2 days. For study of the effects of nefazodone on the hERG channel protein trafficking and protein expression, the hERG-HEK cells were incubated for 24 h with nefazodone (10, 100, 300 nM, and 1 μM).

Table 1
Action potential parameters of hiPSC-CMs.

Cell type	MDP (mV)	dV/dt_{max} (V/s)	APD_{90} (ms)	TA (mV)
Nodal	−56.9 ± 1.2	5.7 ± 0.4	244.4 ± 11.9	85.7 ± 1.4
Atrial	−66.7 ± 0.9	18.4 ± 2.1	284.2 ± 6.9	106.6 ± 1.2
Ventricular	−66.0 ± 0.8	44.5 ± 2.8	492.1 ± 15.4	107.9 ± 0.8
hVMs[a]	−81.8 ± 3.3	215 ± 33	351 ± 14	106.7 ± 1.4

The action potential parameters of three different subtypes are summarized (mean ± SEM). hVMs, human ventricular myocytes.
[a] Magyar et al. (2000).

2.2. Isolation of rat ventricular myocytes (rVMs)

To compare the nefazodone sensitivity on I_{Ca} between rat CMs and hiPSC-CMs, we isolated single rVMs using the Langendorff system. Briefly, the hearts were rapidly excised from anesthetized Sprague–Dawley rats (250–350 g) and perfused via the aorta on a Langendorff apparatus with an oxygenated normal Tyrode's (NT) solution for 5 min to clear the blood, then perfused with Ca^{2+}-free NT solution for 3 min. Next, the heart was perfused with an enzyme solution containing 0.6 mg/ml collagenase (Worthington, type 2, USA) for 8–10 min. Finally, this enzyme-containing solution was washed out for 5 min with a high-K^+ and low-Cl^- Kraft-Bruhe (KB) solution. Following the isolation procedure, the left ventricle was dissected and agitated mechanically with a fire-polished Pasteur pipette in KB solution to obtain single myocytes. The isolated myocytes were stored at 4 °C for 12 h.

2.3. Whole-cell patch clamp recordings in hiPSC-CMs

The cells were cultured for 4 weeks and used at 7 to 28 days post-thaw for electrophysiological analysis. Whole-cell hiPSC-CM recordings were performed at 37 °C using an external solution containing (mM) 145 NaCl, 5.4 KCl, 10 HEPES, 1 $MgCl_2$, 5 glucose, 1.8 $CaCl_2$ (pH 7.4). The internal solution contained (mM) 120 K-Asp, 20 KCl, 5 NaCl, 2 $CaCl_2$, 10 HEPES, 5 EGTA, and 5 Mg-ATP (pH 7.25). We recorded typical APs in hiPSC-CMs in the current-clamp mode. The spontaneous beating activity of single hiPSC-CMs was recorded, and only hiPSC-CMs that could beat stably were included in the analysis. Following stabilization of the AP waveforms, the average of five recorded APs for each test

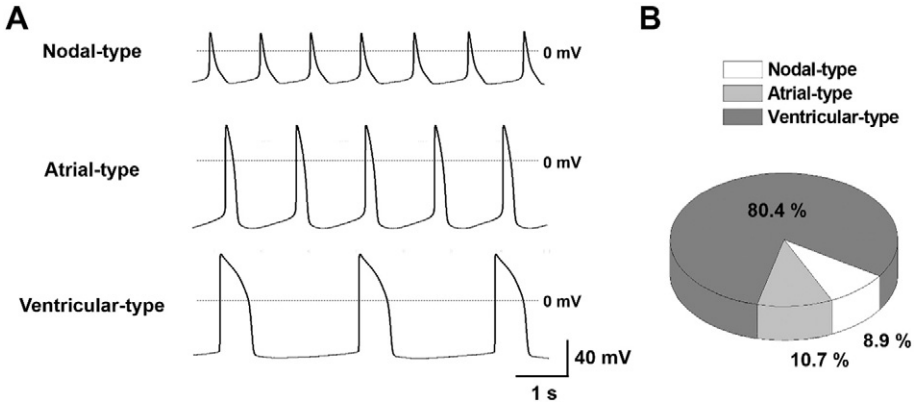

Fig. 1. *Action potential characterization of hiPSC-CMs.* A, representative traces display nodal-type (top), atrial-type (middle), and ventricular-type (bottom) APs in hiPSC-CMs. The cardiac AP subtypes are determined based solely on AP morphology with longer AP duration of ventricle-type cells (APD_{90} > 300 ms) compared to both atrial- and nodal-type cells (APD_{90} between 200 and 300 ms). The distinction between atrial- and nodal-type APs is made by differences in amplitude (TA) and upstroke velocity (dV/dt_{max}). B, comparison of the relative abundance of three different AP subtypes in hiPSC-CMs.