Journal of Pharmacological and Toxicological Methods 91 (2018) 18–26

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

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

Research article

Response of human induced pluripotent stem cell-derived cardiomyocytes to several pharmacological agents when intrinsic syncytial pacing is overcome by acute external stimulation

Haoyu Zeng*, Bharathi Balasubramanian, Armando Lagrutta, Frederick Sannajust

Merck & Co., Inc., Safety & Exploratory Pharmacology Department, West Point, PA, USA

ABSTRACT

We challenged human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) syncytia, mainly, CDI iCells with several classes of well-characterized pharmacological agents (including hERG blocker, Nav1.5 blocker, Cav1.2 blocker and opener, β-adrenergic agonist, and I_f blocker) under pacing conditions, utilizing the Cardio-ECR instrument, a non-invasive platform featuring simultaneous and continuous measurement of synchronized beating rate and contractility (both signals were acquired simultaneously and well aligned). We found that: 1) with increasing acute stimulation rates (no pacing; 1, 1.5, and 2 Hz), beat interval was gradually shortened mainly in the relaxation phase of each beat cycle; 2) typical responses of iCells hiPSC-CMs to all tested pharmacological agents were either attenuated or even eliminated by pacing, in a concentration- and stimulation rate-dependent manner; and 3) when iCells were influenced by pharmacological agents and cannot follow pacing rates, they still beat regularly at exactly 1/2 or 1/3 of pacing rates. We concluded that when intrinsic syncytial pacing was overcome by faster, external stimulations, beat intervals of hiPSC-CMs were mainly shortened in the relaxation phase, instead of proportionally in each beat cycle, with increasing pacing rates. In addition, in response to pharmacological agents upon pacing, hiPSC-CMs exhibited distinct patterns of refractoriness, manifested by skipped beats in pacing-rate dependent manner, and attenuation (or even abolition) of the typical response evoked under spontaneous beating.

1. Introduction

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have emerged as critical tools in an integrated cardiac safety evaluation of drug candidates with demonstrated correlation and predictability to clinical data in several cases (Ando et al., 2017; Kitaguchi et al., 2017; Kopljar et al., 2016; Lagrutta et al., 2016, 2017; Lu et al., 2015; Zeng, Roman, Lis, Lagrutta, & Sannajust, 2016; Zhang et al., 2016), despite exhibiting certain properties different from adult human cardiomyocytes ((Ma et al., 2011; Sala et al., 2016; van Meer, Tertoolen, & Mummery, 2016), and internal unpublished observations). These studies relied on the intrinsic beating rate of the hiPSC-CMs in syncytia, with poorly understood pacemaking mechanisms (unpublished observation, and data shown in this study). By comparison, *in vivo* mature human cardiomyocytes follow a well-defined rate originating from pacemaker cells into the Sino-Atrial node (SAN), to beat with relatively fixed rates. To close this gap, we decided to investigate properties and response of hiPSC-CMs to several reference pharmacological agents under pacing conditions using the well-validated Cardio-ECR instrument (Zhang et al., 2016), a non-invasive platform allowing simultaneous and continuous measurement of synchronized (Fig. 1A) beating rate (i.e., field potential firing rate) and contractility (i.e., impedance amplitude). Due to their distinct properties, several classes of well-known reference pharmacological agents, including hERG blockers such as cisapride (Rampe, Roy, Dennis, & Brown, 1997) and dofetilide (Kiehn, Lacerda, Wible, & Brown, 1996), Nav1.5 blocker flecainide (Ramos & O'leary, 2004), Cav1.2 blocker verapamil (Dilmac, Hilliard, & Hockerman, 2004) and Cav1.2 opener FPL64176 (Jacobo et al., 2009), β-adrenergic agonist isoproterenol (Zhang et al., 2009), and pacemaker blocker ivabradine (Kim et al., 2015) were selected to challenge iCells/hiPSC-CMs for their respective pharmacological responses. We found that when intrinsic syncytial beating was overcome by faster, external stimulations, time intervals of hiPSC-CMs were mainly truncated in the relaxation phase of each beat cycle with increasing pacing rates. In addition, hiPSC-CMs showed attenuated or even eliminated response to pharmacological agents with external stimulations.

* Corresponding author at: Merck & Co., Inc., SALAR-Safety & Exploratory Pharmacology Department, 770 Sumneytown Pike, P.O. Box 4, West Point, PA 19486-0004, USA.
E-mail address: haoyu.zeng@merck.com (H. Zeng).

https://doi.org/10.1016/j.vascn.2017.12.004
Received 26 July 2017; Received in revised form 19 December 2017; Accepted 19 December 2017
Available online 10 January 2018
1056-8719/

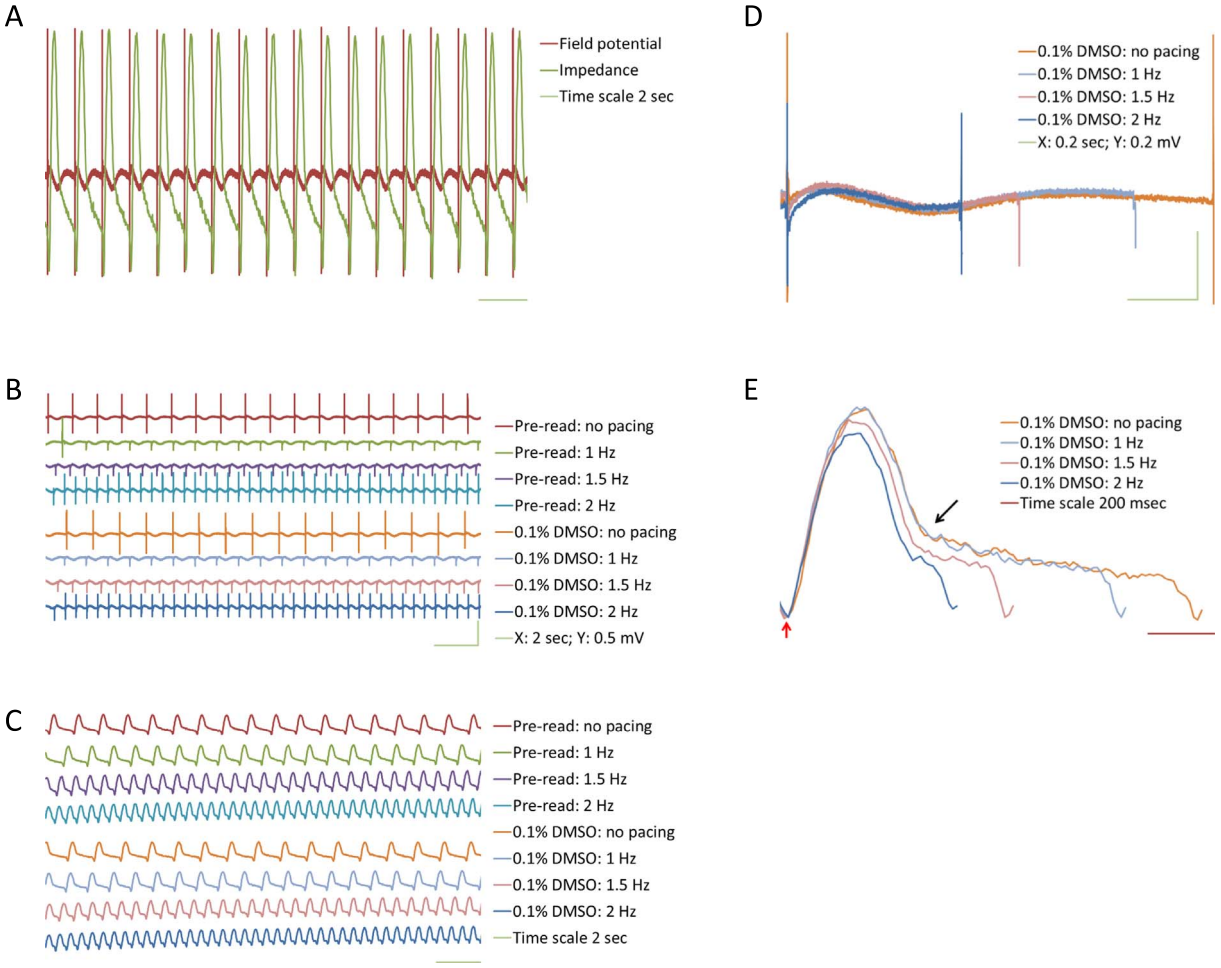

Fig. 1. Representative field potential and impedance traces with vehicle control.
A) A 20-second field potential trace recorded from one well before vehicle addition was superimposed to impedance signal (scaled for illustration) of the same well without alignment; 20-second field potential (B) or impedance (C) traces recorded from that well at different pacing rates before and 1 h after 0.1% DMSO addition as indicated in representative traces; field potential (D) or impedance (E) signals from one beating cycle at different pacing rates were aligned and superimposed. Black arrow points to the feature point in impedance signal, while red arrow indicates the negative peak. The interval between negative peak and feature point is the feature point duration (FtPD) using in data analysis. All field potential traces or impedance traces had the same scale in respective panels.

2. Experimental procedures

The hiPSC-CMs (iCells®) from Cellular Dynamics International (CDI, Madison, WI, USA) were placed onto 48-well Cardio ECR E-Plates® (ACEA Biosciences Inc., San Diego, CA, USA) and cultured for 14 days before use as described previously (Lagrutta et al., 2016). Briefly, cells were seeded to E-plates pre-coated with 10 μg/mL fibronectin (Sigma Aldrich, Catalog# F1141) at 30,000 cells per well. Cells were maintained into incubator at 37 °C with 5% CO_2 and 95% O_2. Culture media (CDI, Madison, WI, USA) were exchanged every 2-3 days, following manufacturer's recommendations.

Test agent (all from Sigma-Aldrich, St. Louis, MO, USA) stock solutions were prepared in 100% DMSO. On the day of experiment, cells were fed with fresh media for at least 3 h in the incubator prior to data acquisition. The plates were then transferred to and read on an xCELLigence® RTCA Cardio ECR instrument (ACEA Biosciences Inc., San Diego, CA, USA). Pre-reads of 1-min were recorded sequentially as controls at respective frequencies (i.e., no pacing, 1, 1.5, and 2 Hz). Stimulation was delivered using built-in square pulses of the instrument with 500 mV voltage and 1 ms duration. This empirical, minimal voltage and duration were selected to avoid baseline shift, and to minimize any potential damage to cells (fold change to threshold was not calculated, since the threshold values were different from well to well). The test agent stock solutions were diluted into media and quickly added to the plate at 1:1000 ratio so the final DMSO concentration in each well was 0.1% (except media wells which contained no additions). After 1-hour incubation in the instrument (hosted in a temperature-controlled incubator), plates were read using identical procedure as control pre-reads. All impedance signals were sampled with 12-ms interval (83.3 Hz sampling rate), while field potential data were collected with 0.1 ms interval (10 KHz sampling rate). All data were analyzed with a built-in analysis software of the instrument and normalized using Microsoft EXCEL®. N = 3 was used in each plate, and three plates were used in the study. All data were normalized to appropriate controls as described in table legends and expressed as: Mean ± SEM. Student's t-test was used for statistical comparison.

3. Results

Following pacing studies by others (Pointon et al., 2015; Qian & Guo, 2010), we challenged hiPSC-CMs with several well-characterized pharmacological agents using Cardio-ECR instrument that simultaneously measured synchronized (Fig. 1A) field potential and contractility with and without stimulations (Fig. 1B and C),in order to explore the properties of iCells/hiPSC-CMs under different external pacing conditions,. We found that Na^+-spike intensity in field potential signals were greatly reduced when paced at 1 or 1.5 Hz (Fig. 1B), and more importantly, the “T” waves in field potential signals (a relatively