Identification of Drug–Drug Interactions In Vitro: A Case Study Evaluating the Effects of Sofosbuvir and Amiodarone on hiPSC-Derived Cardiomyocytes

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ABSTRACT  
Drug–drug interactions pose a difficult drug safety problem, given the increasing number of individuals taking multiple medications and the relative complexity of assessing the potential for interactions. For example, sofosbuvir-based drug treatments have significantly advanced care for hepatitis C virus-infected patients, yet recent reports suggest interactions with amiodarone may cause severe symptomatic bradycardia and thus limit an otherwise extremely effective treatment. Here, we evaluated the ability of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) to recapitulate the interaction between sofosbuvir and amiodarone in vitro, and more generally assessed the feasibility of hiPSC-CMs as a model system for drug–drug interactions. Sofosbuvir alone had negligible effects on cardiomyocyte electrophysiology, whereas the sofosbuvir-amiodarone combination produced dose-dependent effects beyond that of amiodarone alone. By comparison, GS-331007, the primary circulating metabolite of sofosbuvir, had no effect alone or in combination with amiodarone. Further mechanistic studies revealed that the sofosbuvir-amiodarone combination disrupted intracellular calcium (Ca2+) handling and cellular electrophysiology at pharmacologically relevant concentrations, and mechanical activity at supra-pharmacological (30x Cmax) concentrations. These effects were independent of the common mechanisms of direct ion channel block and P-glycoprotein activity. These results support hiPSC-CMs as a comprehensive, yet scalable model system for the identification and evaluation of cardioactive pharmacodynamic drug–drug interactions.  

Key words: cardiomyocyte; cardiac electrophysiology; stem cell; safety; drug interactions.

INTRODUCTION  
Drug–drug interactions pose a difficult drug safety problem, given the increasing number of individuals taking multiple medications (Hajjar et al., 2007) and the relative complexity of assessing the potential for interactions (Prueksaritanont et al., 2013). Although investigations into pharmacokinetic drug–drug interactions are now routine in vitro and in clinical trials (Bjornsson et al., 2003), pharmacodynamic drug-drug interactions...
interactions are impractical to evaluate in clinical trials and generally undetectable with reduced in vitro preparations designed for high throughput screening.

Within cardiovascular safety pharmacology, a long history of drug–drug interactions exists for cardiovascular drugs (Hager et al., 1979; Nutescu et al., 2011; Anderson and Nawarskas, 2001). Indeed, the high degree of polypharmacy (Hajjar et al., 2007), and cardiovascular drug usage (Gurwitz et al., 2003), in the elderly has led to a significant issue with cardiovascular drug–drug interactions (Köhler et al., 2000; Straubhaar et al., 2006). Recent reports have utilized statistical models in an attempt to predict potential drug–drug interactions with electrophysiological cardiac effects (Lorberbaum et al., 2016), but the ability to reproduce, and ultimately screen for, pharmacodynamic drug–drug interactions in vitro has not been demonstrated.

Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) may provide a model for assessing cardiac drug–drug interactions, as these cells are amenable to high throughput in vitro investigations and recapitulate key features of human cardiac electrophysiology. Robust evaluation of potential drug-induced cardiac safety liabilities, created by altered electrophysiology and mechanical activity, can be achieved through a variety of functional hiPSC-CM assays (Doerr et al., 2015; Harris et al., 2013; Guo et al., 2013; Glibchrist et al., 2015). In addition, the comprehensive nature of hiPSC-CMs may enable further mechanistic insight into known or anticipated drug–drug interactions with cardiac effects.

The drug–drug interaction between sofosbuvir and amiodarone serves as a recent example of an unanticipated interaction with cardiac effects. Sofosbuvir-based drugs have significantly advanced care for hepatitis C virus-infected patients (Lawitz et al., 2013), recent post-marketing reports indicate that severe symptomatic bradycardia can occur through the transporter (Soriano et al., 2013), and cardiovascular drug usage (Gurwitz et al., 2013) in the elderly has led to a significant issue with cardiovascular drug–drug interactions (Lawitz et al., 2013), and the minimum effective plasma steady-state concentration of amiodarone, 0.57 μmol/l (Latini et al., 1984). All error bars represent standard deviations.

**Materials and Methods**

The individual and combinatorial effects of sofosbuvir and amiodarone on hiPSC-CMs were evaluated using MEA, impedance (IMP), and calcium imaging (CaI) assays. The iCell Cardiomycytes² (Cellular Dynamics International; CDI) were used for all cardiomyocyte-based assays in this study. All cell and instrument handling was done per manufacturer’s instructions, as described below. Drug doses were based on the reported C_{max} value for sofosbuvir, 1.14 μmol/l (Lawitz et al., 2013), and the minimum effective plasma steady-state concentration of amiodarone, 0.57 μmol/l (Latini et al., 1984).

**Automated Patch Clamp**

**Cell Line Culture**

The overexpressed ion channel cell lines (Cav1.2/β2/α2δ-CHO, no. CT6004; Nav1.5 CHO, no. CT6007; Kv11.1 (hERG) HEK293, no. CT6001, all from Chantest) were cultivated as previously described (Becker et al., 2013; Bruggemann et al., 2009; Obergrussberger et al., 2014). In brief, cells were cultured in T75 culture flasks in manufacturer recommended media and passaged at 50-80% confluency (every 2–3 days) to ensure a healthy suspension of completely dissociated cells prior to recording. Cells were harvested as described previously using trypsin, other suitable enzymes, or even enzyme-free detachment protocols (Becker et al., 2013; Obergrussberger et al., 2014; Bruggemann et al., 2009). Cells were then re-suspended in a mixture of 50% culture media/50% external recording solution at a density of 50,000–500,000 cells per ml.

**Patch Clamp Solutions**

Internal solution for hERG: 50 mmol/l KCl, 10 mmol/l NaCl, 60 mmol/l KF, 20 mmol/l EGTA, 10 mmol/l HEPES/KOH, pH 7.2. Internal solution for Nav1.5 experiments: 10 mmol/l CsCl, 110 mmol/l CsF, 20 mmol/l EGTA, 10 mmol/l HEPES/CaOH, pH 7.2. Internal solution for Cav1.2 experiments: 120 mmol/l CsF, 20 mmol/l KCl, 10 mmol/l NaCl, 2 mmol/l MgCl, 2 mmol/l EDTA, 5 mmol/l EGTA, 10 mmol/l Hepes, pH7.2, 1 mmol/l Na-ATP, 25 μmol/l/Esicn. External recording solution for Nav1.5 and hERG: 140 mmol/l NaCl, 4 mmol/l KC1, 1 mmol/l MgCl2, 2 mmol/l CaCl2, 5 mmol/l D-Glucose monohydrate, 10 mmol/l HEPES/NaOH pH 7.4. External solution for Cav1.2: 80 mmol/l NaCl, 60 mmol/l NMDG, 4 mmol/l KCl, 2 mmol/l CaCl2, 1 mmol/l MgCl2, 5 mmol/l Glucose, 10 mmol/l HEPES, pH 7.4.

**Electrophysiology**

All cells were recorded in the whole cell patch clamp mode using the Patchliner or the SyncroPatch 384P (Nanion Technologies) incorporated into a Biomek FX pipetting robot (Beckman Coulter). hERG recordings were performed at 35 °C, all other recordings at room temperature. Cells were added to the patch clamp recording chips and attached to the aperture of each well by suction when necessary. Voltage protocols were constructed and data acquired using PatchMaster (HEKA Elektronik) or PatchControl 384 (Nanion Technologies).

**Microelectrode Array**

**Cell Culture**

iCell Cardiomycytes² were used for the microelectrode array (MEA) recordings acquired using the Maestro multiwell electrophysiology platform (Axion BioSystems, Inc.). Cells were plated according to cell manufacturer recommendations. Briefly, a 5 μl drop of fibronectin was applied to the electrode array of each well and allowed to incubate for one hour at 37 °C. Cardiomycytes were thawed, centrifuged, and resuspended to 10⁶ cells/ml. The fibronectin was then aspirated from each well.
and replaced with a 5μl drop of the cell suspension (~50k cells/well). The plate was incubated for one hour at 37°C and then 300μl of iCell Maintenance Media (iCMM) added to each well.

**Experimental Protocol**

The media was changed at least two hours prior to the experiment to minimize the effect of thermal, mechanical, or chemical perturbations on the cells. On the day of the experiment, the cell culture plate was moved directly from the incubator to the MEA device for a baseline recording (30 minutes), with environmental controls (37°C and 5% CO2) used to maintain the temperature and pH.

A single dosing scheme was utilized, such that each MEA well received only one dose of a particular compound or compound mixture. Compounds were prepared on the day of use at 10X the final concentration, stored in at 37°C and 5% CO2 until the dosing (no more than 4 hours after formulation). Wells were dosed by removing 10% of the media volume and replacing with the same volume of the prepared compound (at 10X the final concentration). The MEA wells were dosed in a biosafety cabinet, restored to the MEA device, environmental controls re-engaged, and allowed to equilibrate for ~30 minutes. Spontaneous cardiac electrical rhythms were recorded continuously during the equilibration period.

**Data Analysis**

Three primary endpoints were derived from the cardiac field potential (FP) in the baseline and post-dose conditions: 1) spike amplitude (AMP), 2) field potential duration (FPD), and 3) beat period (BP). An example of the cardiac field potential, and the associated measurement definitions, is shown in Supplementary Figure 1. The onset of cardiac depolarization is marked by a sharp deflection in the field potential signal, termed the depolarization spike. Depolarization spike AMP provides a measure of the speed of depolarization in the cardiomyocyte network. FPD is given by the timing interval between the depolarization spike and the peak of the repolarization feature. BP was defined as the duration between two consecutive depolarization spikes.

The baseline and post-dose measurements were taken during the 5 minutes immediately following the equilibration period. All analysis was performed using the Axion Integrated Studio (AxIS) software suite (Axion BioSystems, Inc) where an algorithm used BP to identify the most stable continuous string of 30 beats. These beats were then used to compute the average AMP, FPD, and BP metrics. Baseline and post-dose FPD measurements were rate corrected (FPDc) using the Fridericia correction (Fridericia, 1920); subsequently, the percent change from baseline to post-dose was calculated for each well.

**Optogenetic Pacing**

For the pacing experiment, the cells were transduced by adding 9μl of the viral vector construct (AAV9-ChR2-CAG-GFP, UNC Viral Vector Core) to a cell suspension prepared for 24-wells before seeding the cells on the MEA as described above. The pacing experiment was performed 12 days after transduction to allow sufficient time for expression of the light sensitive channel (opsin). The cells were paced using 5ms pulses of blue light (470nm) from a multiwell light delivery device (Lumos, Axion BioSystems, Inc). Measurements were made after the FPD had stabilized at the paced beating rate.

**Calcium Imaging**

**Cell Culture**

Cryopreserved iCell Cardiomyocytes were thawed and cultured according to the manufacturer recommended protocol with cardiom yocytes seeded at a density of ~16,000 live cells per well in 33μl of iCell CM plating medium (iCMM) in black 384-well plates (Greiner Bio-one) coated with 0.1% gelatin. After 4hr, iCMM was exchanged to 40μl of iCMM with media changes every other day until the day of the assay.

**Experimental Protocol**

On Day 7 post-thaw, media was aspirated and replaced with 20μl of iCMM and cells were loaded with 20μl of the 2X calcium dye from the EarlyTox Cardiotoxicity Kit (Molecular Devices) for 2h in the incubator. Cells were treated with 10μl of 5x compound titrations (or DMSO control) prepared in iCMM and further incubated for 30 minutes. Assay plates were then read on the FDSS/μCell at 37°C.

**Data Analysis**

The amplitude of the fluorescence signal was computed by automated software (Hamamatsu) for each well on the plate, and then the percent change from baseline to post-dose was calculated before averaging across replicate wells.

**Impedance**

**Cell Culture**

iCell Cardiomyocytes were seeded at a density of 50,000 cells per well on a CardioExcyte 96 sensor plate (Nanion Technologies) according to cell manufacturer recommendations. The 96-well sensor plates were coated with fibronectin (Sigma Aldrich; 1:100 solution in phosphate-buffered saline without Ca2+ /Mg2+) and kept for 1.5 h in the incubator (37°C, 5% CO2). A cell suspension of 500 viable cells/μl was prepared from thawed iCell Cardiomyocytes. After removing the fibronectin solution, 100μl pre-warmed 37°C iCMM and then the cell suspension were added to each well. The sensor plate was incubated at 37°C, 5% CO2 for 24hr. After that, the medium was exchanged every 2 to 3 days with iCMM.

**Experimental Protocol**

The medium was changed at least 2 h prior to the experiment to minimize artifacts. A single dosing scheme was applied, such that each well received only one dose of a compound. Compounds were prepared in medium containing 0.3% DMSO on the day of the experiment at 2x the final concentration and kept at 37°C until use. Wells were dosed under sterile conditions by removing 50% of the medium and replacing it by the same volume of the compound (at 2x the final concentration). The sensor plates were returned to the incubator immediately after dosing. Data were recorded continuously until 240 minutes post-dosing. Sampling rate was 1kHz for impedance and 10kHz for field potential (FP), and sweep lengths were 10s.

**Data Analysis**

The software calculated the average beat shape based on the data of one sweep, termed the Mean Beat, which was used for the analysis of signal amplitudes (peak-to-peak amplitude for impedance signals; peak-to-peak amplitude of depolarization spike for FP signals).
RESULTS

**Sofosbuvir Exerts an Electrophysiological Effect in Combination With Amiodarone In Vitro**

The individual and combinational effects of sofosbuvir and amiodarone on hiPSC-CMs were assessed via extracellular MEA experiments (see Methods, Supplementary Figure 1). Figure 1 shows the effect on the raw field potential (FP), spontaneous beat period (BP), field potential duration (FPD), corrected field potential duration (FPDc, Fridericia correction), and depolarization amplitude (AMP).

The raw FP traces in Figure 1A qualitatively illustrate that the vehicle control (0.2% DMSO) and sofosbuvir alone (31.6 μmol/l) at ~30x C\text{max} were without effect. Dose-dependent changes are quantified in Figure 1B, where sofosbuvir alone did not have a significant effect on BP, FPD, FPDc, or AMP across the tested concentrations, which extend 1.5 orders of magnitude above the clinical C\text{max} (Figure 1B).

Amiodarone (0.6 μmol/l) produced an expected prolongation in the raw FP trace (Figure 1A), whereas the sofosbuvir-amiodarone combination (31.6 μmol/l sofosbuvir, 0.6 μmol/l amiodarone) elicited a marked change in the FP trace. The FP metrics were quantified for the amiodarone-sofosbuvir combination using the same escalating concentrations of sofosbuvir with a fixed concentration of amiodarone (Figure 1C). Amiodarone alone (0.6 μmol/l) prolonged BP by 15.0 ± 6.3%, FPD by 18.7 ± 3.9%, and FPDc by 13.3 ± 2.3%, whereas AMP was reduced by 61.0 ± 15.9%, as indicated by the horizontal bars. Sofosbuvir addition eliminated, and then reversed, the effects of amiodarone in a concentration-dependent manner, with significant changes beginning at the clinical C\text{max}. At the highest concentration of sofosbuvir, the drug combination shortened BP by 49.2 ± 5.1%, FPD by 52.9 ± 5.7%, and FPDc by 41.0 ± 5.3% compared to baseline, although causing a 13.3 ± 16.6% increase in AMP (* denotes P < 0.05 between amiodarone and sofosbuvir-amiodarone doses, n = 4, Wilcoxon rank-sum test).

The electrophysiological effects of the drug combination remained over chronic exposures, but recovered after wash-out (Supplementary Figure 2). Quinidine, another P-gp inhibitor like amiodarone, did not interact with sofosbuvir in the MEA assay, nor did amiodarone alter sofosbuvir uptake into cardiomyocytes according to mass spectrometry measurements (see Supplementary Results).

**GS-331007, the Predominant Metabolite of Sofosbuvir, Does Not Interact With Amiodarone In Vitro**

GS-331007 is the primary metabolite of sofosbuvir and accounts for the majority of systemic exposure following a single oral dose of sofosbuvir (Gilead Sciences Inc., 2013). Importantly, however, the metabolism of sofosbuvir occurs in the liver, and thus should not occur with hiPSC-CMs in vitro. Therefore, to ensure this study examined the relevant chemical structure, GS-331007 and amiodarone were evaluated using the MEA assay. The results, illustrated in Figure 2, indicate that GS-331007 does not impact hiPSC-CM electrophysiology when delivered alone, or in combination with amiodarone, at any tested concentration (0.1x – 10x C\text{max}). None of the endpoints showed a significant change from baseline at any concentration of GS-331007 applied to the cells (Figure 2B), consistent with the example trace in Figure 2A. The addition of GS-331007 with amiodarone did not affect BP, FPD, or FPDc beyond using amiodarone alone (Figure 2C). At the highest supra-physiologic concentrations of GS-331007 (3x and 10x the C\text{max}) the drug combination produced a subtle, but statistically relevant, recovery in AMP, as compared to amiodarone alone (n = 4, P < 0.05, Wilcoxon rank-sum test).

**The Sofosbuvir-Amiodarone Combination Shortens Field Potential Duration Independent of Beating Rate**

The cardiomyocytes were paced to isolate changes in repolarization from changes in the spontaneous beating rate. A light sensitive ion channel, channelrhodopsin-2 (ChR2), was targeted to the cardiomyocytes, as described above, to allow optical pacing of the cardiomyocytes (Bruegmann et al., 2010). An example of the onset of pacing is shown in Figure 3A. The BP was immediately entrained by the blue light pulse stimulus (Figure 3A)
and the FPD slowly approached a new steady state value at the paced rate. Measurements of FPD were extracted after 3 minutes of continuous pacing to ensure stabilization. All wells were paced to a BP of 500ms because the sofosbuvir and amiodarone combination decreased the spontaneous BP to 700ms, and cardiomyocytes cannot be entrained to a BP that is longer than the spontaneous BP.

Example paced FP waveforms are shown in Figure 3B, with the results summarized in Figure 3C. All paced results were similar to those obtained from spontaneous FP waveforms. Sofosbuvir did not produce a significant change in FPD (0.2±/-5.7%, n=4, Wilcoxon rank sum test) compared to the vehicle control (-4.7±/-2.4%, n=4) when paced at 2Hz. Amiodarone caused a significant prolongation in FPD (9.2±/-7.4%, n=4, P<0.05, Wilcoxon rank sum test), whereas the sofosbuvir and amiodarone combination significantly reduced FPD (-21.3±/-7.8%, n=4, P<0.05, Wilcoxon rank sum test) in the paced condition.

**The Effect of Sofosbuvir With Amiodarone Is Not Mediated by Block of Potassium, Sodium, or Calcium Channels**

Amiodarone is known to block potassium (I_Kr), sodium (I_Na and I_Na,Late), and calcium (I_CaL) currents, consistent with the
prolongation of FPD and reduction in AMP observed in these and other MEA experiments (Gilchrist et al., 2015). The combination of amiodarone and sofosbuvir shortened FPD and BP, a phenotype commonly observed with \( I_{\text{CaL}} \) blockade (Clements and Thomas, 2014; Harris et al., 2013; Braam et al., 2010). AFC experiments with simple overexpression cell lines were used to determine if direct ion channel block underlay the electrophysiological effects of sofosbuvir-amiodarone.

Figure 4A–C depict raw voltage clamp traces and summarized data for cells overexpressing channels encoded by (A) hERG, (B) Nav1.5, and (C) Cav1.2. The raw waveforms illustrate the effects of the sofosbuvir-amiodarone combination over those of amiodarone alone. Specifically, amiodarone alone (0.5 μmol/l) caused 96 ± 3% block of \( I_{\text{Kr}} \), 22 ± 5% block of peak \( I_{\text{Na}} \), and 12 ± 9% block of \( I_{\text{CaL}} \). In combination with amiodarone, sofosbuvir at 31.6 μmol/l (30xCmax) did not further impact the effects of amiodarone on \( I_{\text{Kr}} \) or \( I_{\text{Na}} \), and showed a statistically relevant, but small, increase in \( I_{\text{CaL}} \) block (\( P < 0.05 \), n = 13–24, unpaired t-test) at only one of four amiodarone concentrations tested (Figure 4D).

Intracellular Calcium Handling Is Impaired by Sofosbuvir and Amiodarone in hiPSC-CMs

Shortening of the field potential duration is commonly associated with direct \( I_{\text{CaL}} \) block, and yet the drug combination did not show a clear \( I_{\text{CaL}} \) block in the automated patch clamp experiments. \( I_{\text{CaL}} \) and the plateau phase of the cardiac action potential may also be affected by alterations in intracellular calcium handling (Bers, 2002). Therefore, the effects of sofosbuvir and amiodarone on intracellular calcium handling were evaluated using high throughput calcium imaging.

In Figure 5A, the raw calcium transient traces show minimal effect of DMSO (0.1%) and sofosbuvir (3 μmol/l) and a modest effect of amiodarone (0.5 μmol/l) on intracellular calcium handling (Figure 5A). Sofosbuvir by itself continued to have no effect over a wide range of doses (Figure 5B), but, in the presence of amiodarone, caused a marked dose-dependent decrease in the intracellular \( Ca^{2+} \) transients at clinical concentrations, and virtually complete elimination of the transient at supra-physiologic concentrations greater than or equal to 10 μmol/l (Figure 5C).

The Sofosbuvir and Amiodarone Combination Disrupts Excitation-Contraction Coupling In Vitro

Decreases in cardiomyocyte intracellular \( Ca^{2+} \) are commonly associated with downstream consequences on the mechanical activity (Bers, 2002). We evaluated this possibility by monitoring the physical movement of the cardiomyocytes with IMP measurements (Guo et al., 2013).

As expected, supra-physiologic concentrations of the sofosbuvir-amiodarone combination decreased cardiomyocyte mechanical activity over the same time period as the MEA and calcium recordings (Figure 6A), whereas time-matched controls were unaffected (n = 3, Figure 6B). Thirty minutes after the addition of the sofosbuvir-amiodarone combination (31.6 μmol/l sofosbuvir, 0.5 μmol/l amiodarone), the beating frequency increased and the IMP signal decreased. Within 120 minutes, the mechanical activity had completely ceased.

The FP was recorded simultaneously with the IMP measurements, revealing a break in excitation-contraction coupling as the electrophysiological activity remained after the cessation of mechanical activity (n = 3, Figure 6C). Neither amiodarone nor sofosbuvir alone decoupled excitation and contraction.

**DISCUSSION**

Four independent labs demonstrated signal disruption in networked hiPSC-CMs with co-administration of sofosbuvir and amiodarone, supporting the robust nature of the drug-drug interaction and reproducibility of the cellular effect. Sofosbuvir and amiodarone shortened cardiomyocyte FPD and BP and impaired intracellular \( Ca^{2+} \) handling at physiologically relevant drug concentrations, whereas myocyte contraction was virtually eliminated at the highest, supra-physiologic concentration tested. Additional experiments confirmed that these effects were due to sofosbuvir and not GS-331007, the primary circulating metabolite.

A common hypothesis for drug-induced changes in cardiac electrophysiology is direct ion channel block. In this case, experimental evidence with simple heterologous channel expression systems—comprising the likely targets of hERG, Nav1.5, or Cav1.2 channels—did not support this hypothesis for the sofosbuvir-amiodarone effect. Instead, the data shown here suggest a primary effect on intracellular calcium handling at clinically relevant concentrations, which could be due to other cellular mechanisms, such as altered second messenger signaling, membrane depolarization, or disruption of sarcoplasmic calcium release, which then influence electrical activity (Bers, 2002). Thus, further mechanistic information might be achieved with follow-up studies focused on key components of...
intracellular calcium cycling such as the ryanodine receptor (RYR2) or calsequestrin (CASQ2), which are implicated in catecholaminergic polyventricular tachycardia (CPVT) (Postma et al., 2005), or the sodium-potassium ATP-ase, which mediates the effect of cardiac glycosides (Ten Eick and Hoffman, 1969). CPVT and cardiac glycosides are both linked to bradycardia clinically, but CPVT also leads to ventricular tachycardia under adrenergic stimulation and cardiac glycosides exhibit tachycardia in iPSC-derived cardiomyocytes (Guo et al., 2013; Gilchrist et al., 2015).

Irrespective of the exact molecular mechanism, these in vitro results provide compelling evidence for a cardiac mechanism of action. Inhibition of P-glycoprotein (P-gp) mediated-drug transport is a common cause of drug–drug interactions and has been postulated in the case of sofosbuvir and amiodarone co-administration (Back and Burger, 2015; Soriano et al., 2015), where P-gp block would lead to increased intracellular concentrations of the victim drug (sofosbuvir) and underlie the adverse event. Such a mechanism is unlikely in this case as sofosbuvir did not have an effect at concentrations 30-fold higher than C_{max} in combination with other P-gp inhibitors such as quinidine, and did not show increased intracellular concentrations in the presence of amiodarone as determined by mass spectrometry studies.

Although the combination of amiodarone and sofosbuvir demonstrated a clear and reproducible effect in vitro, a preliminary interpretation of the data shows a significant deviation from the clinical observations. However, viewing the in vitro and clinical data in the context of their respective underlying systems biology reveals concordant mechanisms and enables relevant translation of the experimental data. The MEA results revealed increased beat rate and shortened field potential duration at physiologic concentrations, whereas all nine reported clinical cases presented as bradycardia, with one patient entering fatal cardiac arrest (FDA, 2015). These seemingly disparate
drug-induced phenotypes may be explained by examining the effects of reduced Ca\(^{2+}\) function under native and in vitro conditions.

Cardiac activity under normal, native conditions in the intact heart is driven by Ca\(^{2+}\) channel-mediated depolarization and conduction in the spatially-segregated sinoatrial (SA) and atrioventricular (AV) nodes (Katz, 1993). Nodal activity is then spread to, and determines the rate of, electrical activity in downstream atrial and ventricular cells. Reduced Ca\(^{2+}\) channel activity under these conditions slows nodal depolarization and electrical conduction and can thus lead to bradycardia in downstream atrial and ventricular cells (DeWitt and Waksman, 2004; Katz, 1993). hiPSC-CM monolayers, on the other hand, do not have a spatial segregation of the mixed cardiomyocyte-subtypes, such that depolarization and conduction is driven by Na\(^{+}\) channel activity with Ca\(^{2+}\) channel activity primarily influencing the plateau phase of the action potential (Ma et al., 2011). Reduced Ca\(^{2+}\) channel activity under these conditions, thus leads to a shortened action potential duration and tachycardia-like activity (Clements and Thomas, 2014; Harris et al., 2013; Braam et al., 2010). In this way, by taking the differing systems biology into account, mechanistic precedent exists for linking clinical bradycardia to in vitro tachycardia in the context of cellular calcium channel activity. More generally, it is important to consider mechanistic information when assessing the directionality of response in reduced in vitro model systems.

Sofosbuvir-amiodarone combination likely exhibited a pharmacodynamic drug–drug interaction. Adverse drug–drug interactions have clear clinical, financial, and societal consequences and thus illustrate a potential need for better prediction of such effects prior to full market release. The sheer number of pharmaceuticals in current use prohibits clinical investigation of drug–drug interactions, but the throughput of hiPSC-CM assays could enable cost-effective, directed assessment of cardioactive pharmacodynamic drug–drug interactions. For example, it would only require a few plate-based tests to profile a compound of interest against a library of 50 commonly prescribed or targeted drugs in an assay of hiPSC-CM functional electrophysiology with a multiwell MEA platform.

The collective in vitro observations provide specific evidence for a cellular mechanism related to altered cardiomyocyte calcium handling when amiodarone and sofosbuvir are co-administered. This data provides novel information by demonstrating that the sofosbuvir-amiodarone response is independent of common pharmacodynamic and pharmacokinetic interactions on ion channel activity, P-gp function, and metabolite production. Furthermore, this work illustrates how to reconcile seemingly disparate in vitro and clinical results and emphasizes the need to take differing systems biology into account when doing so. Ultimately, these results demonstrate the importance of measuring multiple endpoints within an intact biologically relevant model and, more generally, reinforce the utility of hiPSC-CMs for scalable integrated assessments of cardiac safety liability in vitro.

**SUPPLEMENTARY DATA**

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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