PREDICTING CARDIAC RISK OF ANTI-CANCER DRUGS: A ROLE FOR HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Andrew Bruening-Wright, Leslie Ellison, James Kramer, Carlos A. Obejero-Paz

charles river

1 ABSTRACT

Cardiotoxicity is a major complication of many anti-cancer drugs. Acute effects on cardiac ion channels alter cardiac excitability and induce arrhythmias and ultimately heart failure can develop during chronic treatment. Current in vitro strategies for detecting these risks are minimal and often ineffective, particularly for effects that occur over the course of days or weeks. We aimed to validate a human cell-based assay that is fast, robust, and predictive of both acute and chronic clinical outcomes. Currently marketed chemotherapeutic agents were tested on the CDI-CardioECR system (CDI/Fujifilm-ACEA Biosciences). Cardiomyocytes were exposed for eight days to doxorubicin and various tyrosine kinase inhibitors (erlotinib, lapatinib, nilotinib, sunitinib, and crizotinib) at concentrations comparable to clinical plasma values. Recordings were made at multiple time points each day and data was analyzed using proprietary algorithms written in VSA and Matlab. Drugs with hERG channel block liability (lapatinib, nilotinib, sunitinib and crizotinib) showed a dose dependent delay in repolarization and an increase in dysrhythmic markers. Consistent with the clinical observations, cardiac contractility was decreased by crizotinib, lapatinib, nilotinib and sunitinib. Interestingly, the effect of erlotinib on contraction was only apparent after four days exposure which agrees with a previously reported animal study and human case report. Drugs (e.g. lapatinib) that block L-type calcium channels decrease the associated impedance signal (LTAS) with comparable potency. Time-dependent LTAS decrease beyond the initial response can be in principle ascribed to additional mechanisms including channel trafficking and regulation. This may be the case for sunitinib where concentrations of the compound that minimally block calcium channels have no initial effects on LTAS induce significant decreases of this parameter after one day of exposure. The data indicates that the CDI-CardioECR system is a valuable tool to measure the cardiac excitation-contraction mechanisms targeted by anti-cancer drugs, and can be employed in de-risking strategies prior to clinical use.

2 INTRODUCTION

Cardiotoxicity is a major complication of cancer therapy. It can occur after acute exposure to chemotherapeutic agents by direct effects on cardiac ion channels resulting in derangement of cardiac excitability and arrhythmias or develop with time in the form of ventricular dysfunction and heart failure. Current in vitro strategies for detecting these risks are minimal and often ineffective, particularly for effects that occur over the course of days or weeks. This is particularly important when it is recognized that drugs may have direct effects on the synthesis and transport of channels to the membrane surface, mechanisms with half lives of many hours.

The CDI-CardioECR system allows for the recording of field potential and impedance signals of stimulated or spontaneously beating stem-cell derived cardiomyocytes (SC-CM). The main component of the impedance signal is the impedance twitch (IT, see figure 1), a transient increase in electrode resistance that is obliterated by the myosin kinase inhibitor blebbistatin. Three additional small impedance deflections are detected, two preceding the impedance twitch (P1 and P2) and one occurring during relaxation (P3). We presented evidence that P1 is a L-type calcium channel associated signal (LTAS) (Obejero-Paz et al., 2017, Biophysical Society Meeting).

Here we show the long term effects of 5 anticancer drugs using the CDI-CardioECR system. Acute and long-term effects were interpreted in the context of the acute blocking potencies of the drugs on major cardiac ion channels and known clinical outcomes.

3 METHODS

We used the xCELLigence RTCA CardioECR instrument (ACEA Biosciences) to record impedance and extracellular field potentials. iCell cardiomyocytes[2], from Cellular Dynamics International/FUJIFILM. Spontaneous twitch activity was recorded for two 48 well plates. Analysis was performed using Origin, Matlab and macros written in VBA. The output of the instrument is the Cell Index, a measure of the electrode impedance relative to the background reading. During the analysis values were transformed to ohms according to $Z = CI \times 15\Omega$. Field potential and impedance twitches in each well were detected, time shifted relative to the negative peak of the sodium spike signal (t=0) and averaged. Single well averages where then combined in grand averages that summarize a specific treatment. Blebbistatin at 10 μM was used to block motion dependent impedance changes while maintaining excitability.

iCell cardiomyocytes[2] were exposed to different concentrations of anticancer drugs and the effects on cell excitability and contraction were followed for eight days. To assure a stable drug concentration, the culture medium containing fresh drug was replaced daily. Effects on field potential and impedance parameters were compared with blocking potencies for hERG, Cav1.2 and Nav1.5. Raw data is shown in bar graphics. Changes after drug exposure or in vehicle control were expressed relative to baseline according the following equation Δ parameter (%) = (Parameter @ time=t – Parameter @ baseline)x100 / Parameter @ baseline. The effect of the treatment was obtained after subtracting the changes observed in time matched control according to the following equation: $(\Delta \text{ parameter } (\%)_{treatment} - \Delta \text{ parameter } (\%)_{control})$. The associated error was calculated according the following equation $(SEM_{treatment}^2 + SEM_{control}^2)^{0.5}$.

4 CONCLUSIONS

Acute anticancer drug effects on field potential and impedance parameters are predicted by ion channel blocking potencies. These direct effects are a baseline to which long term effects on channels, likely the consequence of distinct modulatory processes, should be compared. The CDI-CardioECR system detects acute and long-term TKI effects, predicts clinical cardiac risk, and may be a useful tool for rank-ordering and lead selection.

1) The Field Potential and Impedance signals
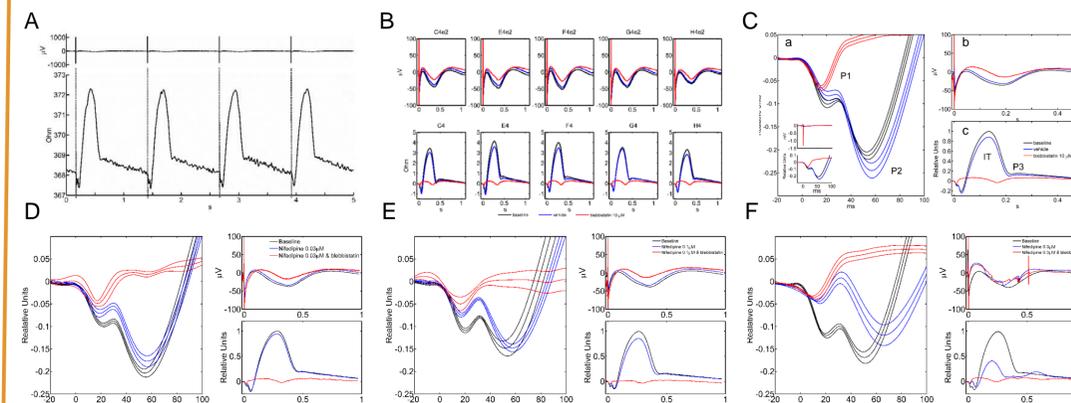
A) Field potential (top) and impedance signals (bottom). Vertical lines define the position of the negative peak of the sodium spike. Twitch activity was synchronized by field stimulation delivered at 0.8 Hz. Stimulus artifacts were removed for clarity. Note the two downward deflections that precede each twitch. **B)** Field potential and impedance local averages from five independent wells. Each average includes 47 field potentials and twitches. Black, blue and red lines indicate signals recorded at baseline, after exposure to vehicle and in the presence of 10 μM blebbistatin respectively. **C)** Grand average of the four local averages shown at two different scales. To compare between wells local averages were normalized with respect to the peak amplitude at baseline. Lines indicate mean ± SEM. a) Magnification of P1 and P2. The inset emphasizes the location of the LTAS P1 with respect to the sodium spike. Note that P2 but not P1 is affected by blebbistatin. b) Field potential grand averages. c) Impedance twitch grand averages. **D-F:** Effect of nifedipine on the field potential and impedance signals.

2) Doxorubicin
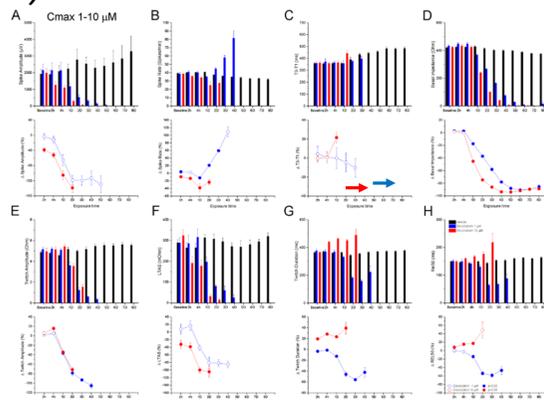
Doxorubicin therapy is limited by the development of a cardiomyopathy that may occur years after treatment cessation. Doxorubicin treatment is associated with apoptotic cell death (Octavia et al., JMCC 52:1213, 2012) and direct effects on calcium homeostasis and the contractile machinery (De Beer et al., EJP, 415:1, 2001). Consistent with these mechanisms doxorubicin decreased basal impedance in a time and concentration dependent manner (D). Notably acute exposure to 10 μM doxorubicin decreased LTAS but have a potentiating effect on twitch peak amplitude (E) suggesting a derangement of the excitation-contraction coupling mechanisms. Arrows in (C) indicate the starting time of dysrhythmic markers.

3) Erlotinib
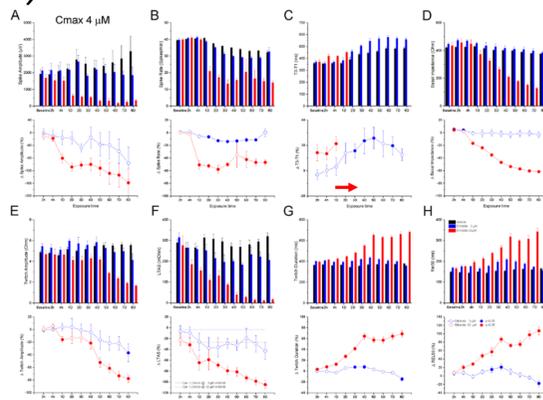
Erlotinib treatment does not effect the QT interval or the left ventricular function but is associated with sudden death with a proportional reporting ratio ≥ 2 and < 3 (Shah & Morganroth, Drug Saf, 38:693,2015). Consistent with a hERG IC50 > 10 μM no acute effect on the repolarizing parameter T3-T1 was observed (blue symbols, C). T3-T1 prolongation was a late event not associated with the decrease in beating frequency (B) since it persists after frequency correction (Fridericia method, not shown). Erlotinib concentrations ≥10 μM decrease cell viability (Doherty et al., TAP 272:245,2013) (D). Dashed lines in (F) indicate the predicted calcium channel inhibition. The arrow in (C) indicates the starting time of dysrhythmic markers.

4) Lapatinib
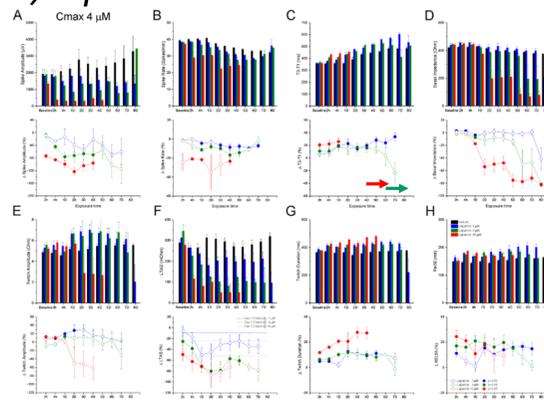
Lapatinib induces QT prolongation, left ventricular dysfunction, sudden death and syncope (Shah & Morganroth, 2015). Lapatinib prolongs the T3-T1 interval consistent with the hERG IC50 of 1 μM (C). Acute exposure to Lapatinib decreases LTAS in a concentration dependent manner consistent with its calcium channel blocking potency (IC50 9.2 μM) (F). However, a late decrease in LTAS remains to be investigated. The effect of lapatinib on the twitch amplitude is complex revealing a dissociation between calcium channel activation and contraction. Lapatinib decreases cell viability in a concentration and time dependent manner (D). Arrows in (C) indicate the starting time of dysrhythmic markers.

5) Crizotinib
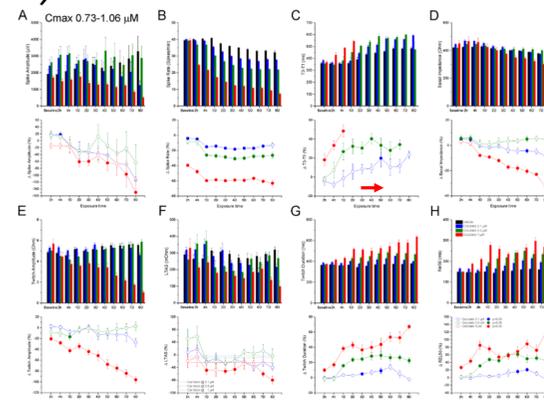
Crizotinib induces QT prolongation and left ventricular dysfunction (Shah & Morganroth, 2015). Crizotinib decreases basal impedance (D) and twitch amplitude (E) in a concentration dependent manner consistent with cell death (Doherty et al., 2013). Consistent with its hERG blocking potency (IC50 1.1-1.7μM), crizotinib prolongs T3-T1. Notably, T3-T1 prolongation at 0.3 μM was observed only after one day exposure suggesting a mechanism distinct from direct channel block. Dashed lines in (F) indicate the predicted calcium channel inhibition. The arrow in (C) indicates the starting time of dysrhythmic markers.

6) Sunitinib
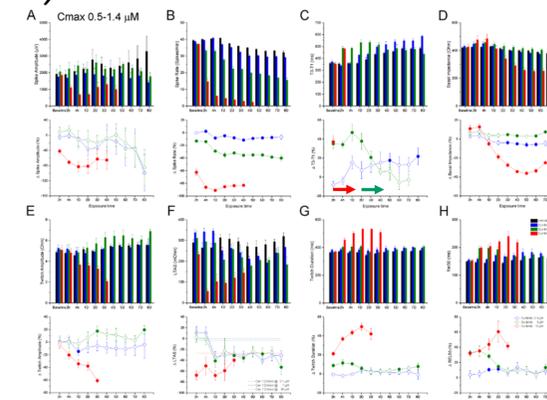
Sunitinib induces QT prolongation and left ventricular dysfunction (Shah & Morganroth, 2015). Sunitinib decreases basal impedance (D) and twitch amplitude (E) in a concentration dependent manner consistent with cell death (Doherty et al., 2013). The delayed increase in basal impedance at 1 μM is consistent with the reported hypertrophic effects (Doherty et al., 2013). Consistent with its hERG blocking potency (IC50 1-2 μM), sunitinib prolongs T3-T1. Notably, T3-T1 prolongation at 0.1 μM was observed only after one day exposure suggesting an additional mechanism distinct from direct channel block. Dashed lines in (F) indicate the predicted calcium channel inhibition. Arrows in (C) indicate the starting time of dysrhythmic markers.