Evaluating drug-induced proarrhythmic risk with CardioECR system

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Outline

• Background
• CardioECR system introduction
• Case studies
• Summary/Conclusion
• Q&A
Basic principle of heart beat

- Cardiac action potentials (AP) starts from pacemaker
- Each phase is contributed by different cardiac ion channels
- E-C coupling leads to cardiomyocyte contraction

CardioECR Webinar | Mar. 11, 2015
Off-target effect examples

Block of $I_{Na}$ ($Na_{V1.5}$) → QRS Prolongation → Ventricular Arrhythmia / Block
Block of $I_{Ca}$ ($Ca_{V1.2}$) → PR Prolongation → Atrio-Ventr. Conduction Block
Block of $I_{Kr}$ (hERG) or $I_{Ks}$ → QT Prolongation → Torsade de Pointes (TdP)
Block of $I_{K1}$ ($K_{ir2.1}$) → QT Prolongation → Ventricular Arrhythmia
Why focus on cardiovascular safety?

• Cardiac toxicity contributes to ~42% toxicity related drug failure or withdrawals
• Drug development of many small molecules was halted, due to off-target adverse effects on cardiac ion channels, and/or other cardiac safety concerns
• hERG channel counter-screening plays a central role in early-stage cardiac safety efforts
Drug withdrawn due to torsadogenic QT prolongation

- Recently withdrawn drugs are all potent hERG channel blockers (a non-exhaustive list)
  - Astemizole
  - Sertindole
  - Sparfloxacin
  - Terfenadine
  - Terodiline
  - Grepafloxacin
  - Cisapride
  - Droperidol
  - Grepafloxacin
  - Levomethadyl
  - Droperidol

- hERG channel is thus the major target for drug-induced QT prolongation

- Currently, the ICH-S7B guideline recommends an *in vitro* hERG evaluation of all pharmaceutical compounds that are targeted for human use

- hERG blockers ≠ QT prolongation ≠ torsadogenic
New development the in vitro cardio toxicity screening

• FDA hosted a workshop on Jul-2013, aiming to change ICH E14/S7B guideline for better assessment of drug-associated proarrhythmia
  – Plan to revise ICH S7B guideline by July 2016
    • To include studies on other cardiac ion channels, in addition to hERG
    • To include studies using human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM)
  – Plan to remove ICH E14 guideline by July 2015
    • Thorough QT study was costly (> 300 patients)
    • Yet, outcome is not very specific to evaluate ventricular pro-arrhythmia risk
      – Non-proarrhythmic factors, such as autonomic perturbations, glucose levels, decrease in body temperature and alterations of circadian rhythms, can prolong QTc

1. Sager et al., 2014
CiPA initiative

• CiPA: Comprehensive In Vitro Pro-arrhythmia Assessment
• It is an initiative responding to FDA’s call for action from the workshop on Jul-2013
• Purpose: to evaluate DIRECT pro-arrhythmic risk of test compounds
• Method: multiple cardiac ion channel data + in silico modeling + studies using hiPSC-CM

How to conduct the studies? How to implement?
  – Part of one proposal is to measure field potential of hiPSC-CM

Sager et al., 2014
hiPS-CM based FP is a surrogate of ECG

Good linear relationship between
1. Rising time of FP and AP
2. Duration of FP and AP

T wave is reflected by $\text{FP}_{\text{max}}$

* "T" in real FP signal is not as obvious as shown here

Original Paper
Cellular Physiology and Biochemistry
Accepted: May 02, 2003

Estimation of Action Potential Changes from Field Potential Recordings in Multicellular Mouse Cardiac Myocyte Cultures
Marcel D. Halbach¹, Ulrich Egert², Jürgen Hescheler¹ and Kathrin Banach¹

Courtesy of ACEA
ACEA CardioECR system

- Monitor cardiomyocytes syncytial beats in 48-well plate format
- Cells in contact with gold electrodes at the bottom of each well
  - Impedance signal generated by applied low-voltage, creating current between electrodes
  - Two field potential electrodes in close proximity to syncytium in each well
- Simultaneous real-time measurement of syncytial contractility by impedance (IMP), and electric activity by field potential (FP) measurement
- High throughput system
- End-points:
  - Impedance
    - **Cell Index** – baseline impedance reflecting syncytial attachment and health
    - **Beat rate** – number of full cycles between beginning of the first detected cycle to the end of the last detected cycle during ~1 min recording
  - **Beat amplitude**
  - Field potential
    - **Field potential spike amplitude**
    - **Field potential rate** – analogous to beat rate
    - **Field potential duration**

Fluctuations Around Impedance Baseline (~1% of baseline)

Cell Index (Baseline)

Full Cycle

Field Potential Spike Amplitude/IMP, fluctuations

Full Cycle

Beat Amplitude

Fluctuations

Baseline Impedance or “Cell Index” Following Plating of iCell hiPSC Cardiomyocytes

Cell expansion phase

stable monolayer synchronously beating “steady state”

Courtesy of A. Lagrutta and J. Imredy
Advantage of CardioECR system

- Non-invasive, continuous recording in cell incubator for days
- Simultaneous and multi-parametric analysis of hiPS-CM *in vitro*
- IMP parameter:
  - Cell index indicates cell attachment/health for cytotoxicity
  - Impedance provides cell contractility data
- FP parameter:
  - Ensemble data of hiPSC-CM membrane electrical activities
  - Integrated readout of drug effects on cardiac ion channels
- IMP signal help to identify and understand FP signal
  - Identification of “T-wave”
- Combination of IMP and FP signals help to correctly understand test compound effect
  - A plus to have multiple parameters for test compound effect
  - In some cases, it is a must to correctly explain test compound effect
    - Cytotoxicity vs. test compound effect that leads to attenuate/disappear of FP spike
    - E-C decoupling vs. conduction block in cessation of IMP signal
Case study

- We conducted a pilot study to evaluate test compound effects on hiPS-CM with FP measurement using ACEA CardioECR system

- The data analysis software, RTCA CardioECR Software 1.0, is fully functional and can quickly and reliably detect and export FP spike amplitude, firing interval and rate, and arrhythmia can be easily identified by visual inspection

- However, the detection/identification of the so-called T-wave in FP by the software is still a challenge. Therefore it is currently handled manually using the RTCA CardioECR software
  - User visually inspects and corrects, when needed, the detection of the T-wave
  - The software then calculates the corresponding FP duration
Case study #1: Moxifloxacin

Stable cell index after test compound addition indicated non-cytotoxicity.

IMP and FP signal were well aligned (mainly slower rates), yet no EAD was observed at 100 µM.
Case study #2: Flecainide

Stable cell index after test compound addition indicated non-cytotoxicity

Pre-read (before cmpd addition)

30 min after cmpd addition

Both rates decreased in the presence of Flecainide. But IMP amplitude was not sensitive to $I_{Na}$ blocker, while FP amplitude clearly demonstrated test compound effect on $Na^+$ spike
Case study #2: Flecainide (zoom in)

Identical X- and Y- axis scales.
IMP AMP reduced ~40%, but FP AMP reduced ~94% in the presence of 3 µM Flecainide.
If only based on IMP signal, the test compound effect will be under-estimated.
Case study #3: Mexiletine

Compared to Flecainide, Mexiletine demonstrated a different case:

Amplitudes of both IMP and FP were down to limit of detection with 30 µM Mexiletine.

We believed the syncytia stopped beating.
Case study #4: Quinidine - T-wave in FP

Full 1 min sweep

Average of all FP wave within 1 min, but where is the peak of “T”??
Case study #4: Quinidine - T-wave detection

If the average FP signal was superimposed to IMP wave…

When IMP signal was superimposed with FP signal, it became clear that the inflection point we picked in FP wave was well-aligned with the inflection point in IMP signal. Therefore, we believed it was the “T”.
Case study #4: Quinidine - QT prolongation

The average FP waves of before and after Quinidine were superimposed

Pre-read

30 min after 0.3 µM Quinidine
Case study #4: Quinidine - EAD

Pre-read

30 min after 1 \( \mu \text{M} \) Quinidine

Arrhythmia (EAD)
Case study #5: Nifedipine

Nifedipine concentration-dependently increased beating rate, and reduced IMP amplitudes, while the FP amplitude was not sensitive to CaV\textsubscript{1.2} channel blocker, just opposite to NaV\textsubscript{1.5} blocker Flecainide.
Case study #5: Nifedipine - QT shortening

The average FP waves of before and after Nifedipine addition were superimposed

30 min after 0.01 µM Nifedipine
QT was shortened by 21%

Pre-read
### Summary of pilot study results

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Conclusion

• Data generated in the pilot study with all eight well-known standards were in line with literature and deemed satisfactory.

• Compared to other MEA systems, the major feature of CardioECR system is to simultaneously record syncytial contractility by impedance, and electric activity by field potential.

• Multiple parameters provide advantage in evaluating test compound effect as shown in the case studies:
  – Cross-reference IMP data to identify T-wave in FP
  – CI data can be used to exclude cytotoxicity
  – FP amplitude is sensitive to Na$_{V1.5}$ blocker
  – IMP amplitude is sensitive to Ca$_{V1.2}$ blocker
  – Disappearance of both FP and IMP signal indicates a true cessation of syncytial beating.
During the pilot study, we found that the FP amplitude had relatively large variation.

As the sampling rate was 10 KHz, high enough to capture Na\(^+\) signal, the variation in Na\(^+\) spike should not be due to under-sampling.

One possible cause could be small changes in the distance at the contact between cells and the recording electrodes?

48-hr continuously monitoring (n=3)
Questions?