

Cardio Specific Toxic Effects of Chemical Warfare Agents

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Objective

Cardio specific toxic effects of chemical warfare agents (CWAs) are being studied as a part of the Edgewood Chemical Biological Center in vitro toxicology programs. This model is being studied to create a non-animal model that can be utilized to study exposures in a way that can be utilized to identify biomarkers for diagnostics and pathway identification leading to enhanced countermeasures. Using stem cell derived cardiomyocytes, we have shown the direct effects of some CWAs on beat rate as well as beat pattern establishing that CWAs do have a direct effect on cardiac function. The mechanisms of toxicity are being characterized using High-Content Analysis (HCA) to identify cellular changes during exposure and Activity Based Protein Profiling (ABPP) to identify protein targets. These efforts are adding in the understanding of CWA toxicity and will lead to better quality of care for the Warfighter.

Methods

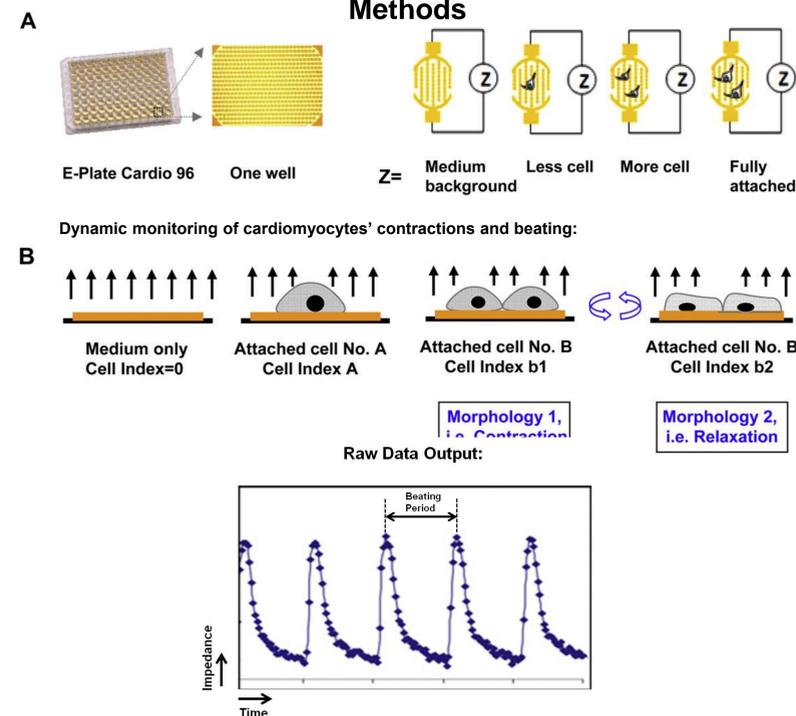


Figure 1. Analysis of Cardiomyocytes. The ACEA Biosciences' xCELLigence Real-Time Cell Analyzer Cardio system utilizing non-invasive impedance readout for real time monitoring of cell viability and functional beating activity of cardiomyocytes. This system can be used to assess the cytotoxicity and cardiotoxicity of CWAs. **A)** The system uses 96-well plates with each well containing a number of electrodes to measure current. **B)** The impedance of the current is directly affected by the concentration and morphology of the cells. The impedance of each well can be measured and recorded at multiple time points. A CO₂ incubator has been modified for the testing of CWAs within engineering controls.

The advantages to this system include: Fast, safe, inexpensive, and validated by big pharma; Real-time analysis, physiologic endpoint, label-free; Medium to high throughput analysis; More predictive than other in vitro tests

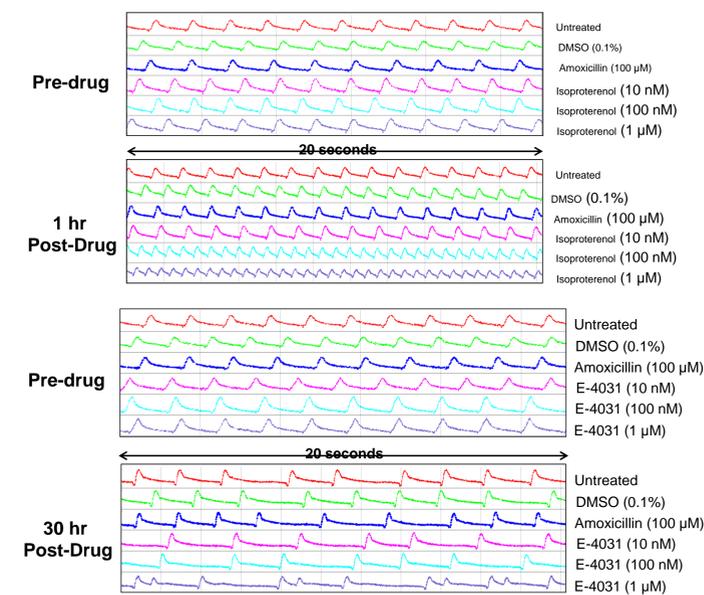


Figure 2. Validation of the Cardiomyocyte Model. Two compounds, isoproterenol and E-4031, with known cardiotoxicity were used to validate our model. Isoproterenol is known to increase heart rate while E-4031 is known to suppress it. Both of these compounds functioned as expected. This confirmed that our model could be used to analyze the direct cardiotoxicity of CWAs.



Results

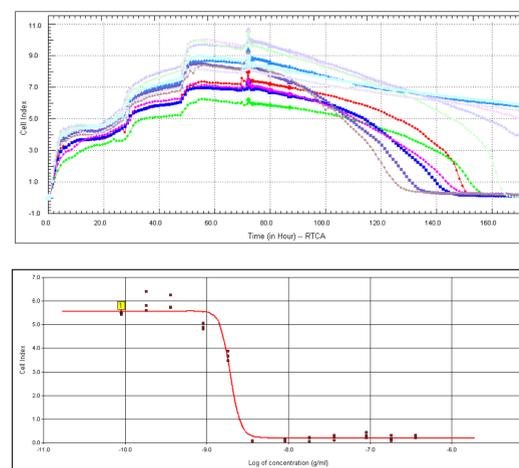


Figure 3. The Direct Effects of Ricin on Cardiomyocytes. The xCELLigence RTCA system was utilized to measure the effects of ricin. The ricin, as expected, killed the cells as shown by the impedance of the wells. This toxicity was titrated out and the IC₅₀ was determined to be 1.9 ng/mL. This also confirmed that our model can be used to measure not only the effects on beating but also direct cytotoxicity.

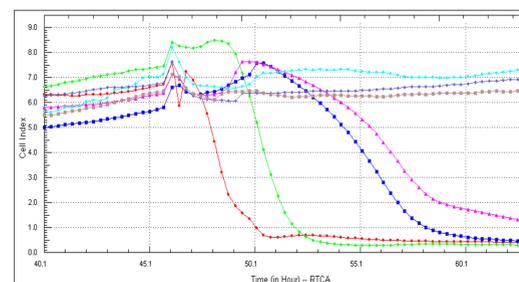


Figure 4. Toxicity of VX on Vero Cells. VERO cells were grown to confluency and treated with 4 different concentrations (10 - Red, 5 - Green, 2.5 - Dark Blue, 1.25 - Purple, 0 - Brown µg/mL) and controls of VX. All four concentrations were shown to be cytotoxic to the cells when compared to the vehicle control. However, as expected, the time required to kill all the cells increased as the concentration decreased.

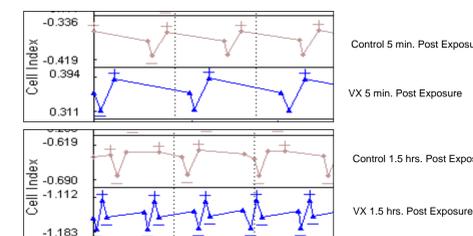
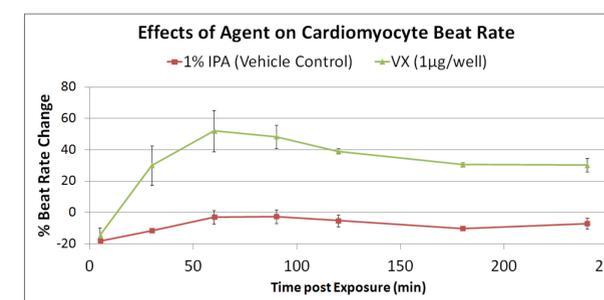


Figure 5. The Effects of VX on Beating Cardiomyocyte Beating. Cardiomyocytes were exposed to VX diluted in 1% IPA and controls and monitored for effects on beat rates and patterns. The VX was shown to not be cytotoxic to the cells. However, toxic effects were seen via the beat rates and patterns. The vehicle control had a slightly negative effect on beat rate compared to the naive control, while the beat rate of cells exposed to VX increased by ~50% 1 hour post exposure. At 20 minutes post exposure an arrhythmia began appearing in the exposed cultures with a maximum variation happening at 1.5 hours post exposure.

Discussion

Cardiac arrhythmia can be a life-threatening medical emergency that may be triggered by xenobiotics including chemical warfare nerve agents and other organophosphate compounds. Little data exists on the cardiovascular effects of nerve agents and many other compounds that are potential threats to the Warfighter, including toxic industrial chemicals and toxic industrial materials (TICs and TIMs).

Currently, a number of methods exist for assessing xenobiotic-induced cardiotoxicity, each with its own limitations. Within the Department of Defense Chemical and Biological Defense Program, cardiotoxicity of compounds of interest are typically assessed by animal testing. The liabilities of animal models include time and expense. In addition, laboratory personnel are placed at risk due to required high doses administered and the uncertainty of interspecies extrapolation. The pharmaceutical industry screens new drug candidates initially for hERG (human ether-a-go-go-related gene) channel inhibition to assess their risk of inducing cardiotoxicity. The hERG channel is an inward rectifying potassium channel, the inhibition of which can lead to prolongation of the time between depolarization and repolarization (i.e. QT interval) and eventually arrhythmia. hERG inhibition assays are typically conducted in vitro with non-cardiac cells over expressing hERG.

Despite being widely used and strongly recommended by regulatory agencies, hERG screening has the following significant limitations: 1) some compounds that inhibit the hERG channel do not induce QT prolongation or arrhythmia; 2) some compounds that do not inhibit hERG in vitro have been shown to induce arrhythmia in humans; 3) some drug compounds have been shown to induce QT prolongation without inducing arrhythmia. Following hERG screening and prior to animal testing, other assays used to assess risk of arrhythmia include Langendorff, Purkinje fiber, and ventricular wedge preparations. These methods are all low throughput ex vivo methods that also share the liability of interspecies variability.

Another technology used to examine the cardiotoxic potential of drug compounds is the microelectrode array (MEA), which can be used to record extracellular electrical activity in excitable multicellular preparations including cardiomyocytes. Changes in field potentials measured by MEAs have been used as a surrogate for arrhythmias, but the inability to detect some proarrhythmic events and the relatively low throughput of the MEA system has limited its acceptance as a test for xenobiotic-induced cardiotoxicity.

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