High-content analysis of a live multiplexed cytotoxicity study using Cytiva™ Cardiomyocytes and IN Cell Analyzer 2000

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GE Healthcare Life Sciences' Cytiva Cardiomyocytes are derived from human embryonic stem cells. In this study, Cytiva Cardiomyocytes were challenged with a panel of test compounds with known or suspected cardiotoxic liabilities. High-content analysis (HCA) of the results allowed cardiotoxic compounds to be identified and distinguished from each other based on their phenotypic profiles.

Introduction

During drug development, early identification and characterization of toxic compounds can help prevent potentially harmful drugs from progressing into costly and time-consuming animal studies or clinical trials. Since cardiotoxicity is a common cause of drug withdrawal during development, there has been an increased demand for more relevant and readily available cell models for *in vitro* cardiotoxicity testing. To address this need, GE Healthcare now offers human cardiomyocytes derived from stem cells, a self-renewing source for scalable manufacture of somatic cells. GE Healthcare's Cytiva Cardiomyocytes constitute a physiologically relevant cell model for early safety testing. Here, the effects of six different test compounds on Cytiva Cardiomyocytes were examined using a multiplexed high-content imaging assay.

Method

Following incubation with test compound, Cytiva Cardiomyocytes were incubated with a cocktail of four fluorescent probes that simultaneously report on plasma membrane integrity, calcium homeostasis, nuclear phenotype, and mitochondrial status. The cells were then imaged live with IN Cell Analyzer 2000, and the images subjected to HCA using IN Cell Investigator software.

Results and discussion

Inspection of representative images from the study (Fig 1) confirmed that there are distinct differences in the cellular phenotypes induced by test compounds such as doxorubicin and amiodarone.

Doxorubicin is a chemotherapeutic agent known to induce acute cardiotoxicity by a mechanism that involves cardiomyocyte apoptosis (1). Doxorubicin toxicity was readily detected by our assay, which reported a dose-dependent decrease in plasma membrane integrity concomitant with dose-dependent changes in nuclear area, mitochondrial shape (1/form factor), and intracellular calcium concentration. The toxic dose inducing half maximal plasma membrane integrity of the sample (TD_{50}) was 1.6 µM, which is the same order of magnitude as values reported with other model systems (2).

Amiodarone is a class III antiarrhythmic drug that has multiple channel blocking actions. Dose-response analysis demonstrated that amiodarone induces loss of plasma membrane integrity with a TD_{50} of 4.5 µM, just above typical therapeutic doses, which can range from 0.75 to 3 µM (0.5 to 2 µg/ml) (3). Amiodarone also affects nuclear area, mitochondrial shape and calcium mobilization, but with pharmacodynamics which differ from those elicited by doxorubicin.

While conventional dose-response plots derived from this four-color toxicity assay indicated differences in compound effects, HCA was able to extract much more detailed and robust information from the images by applying sophisticated analysis techniques to assess more than 50 individual cell measurements collected from every cell imaged in the study. The wealth of measurements amassed by the study was analyzed using clustering and profiling tools accessible through IN Cell Investigator software.

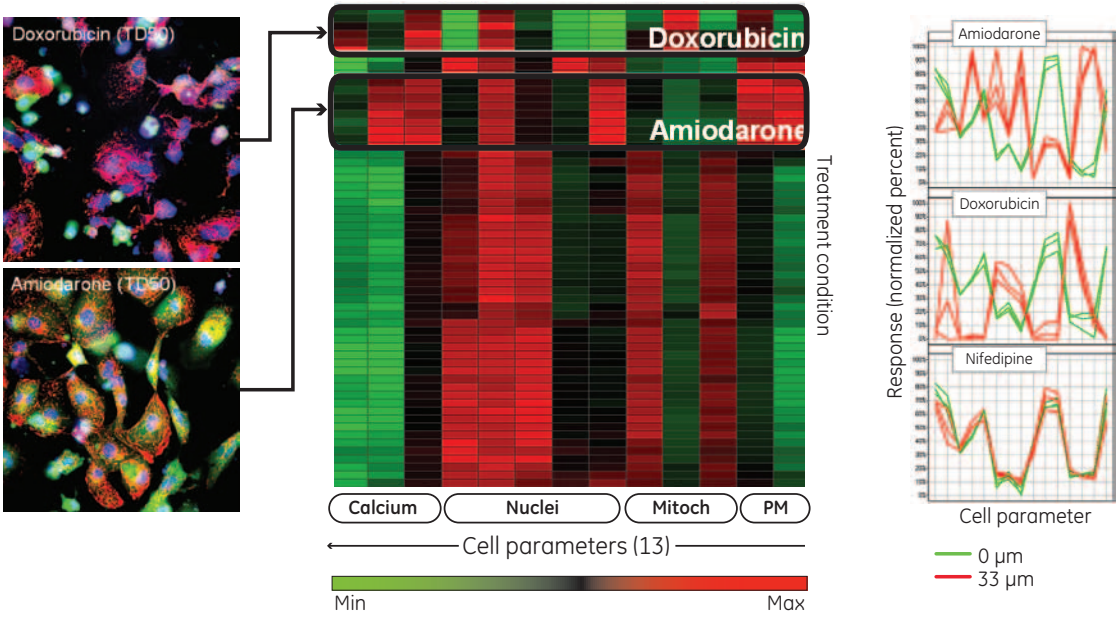
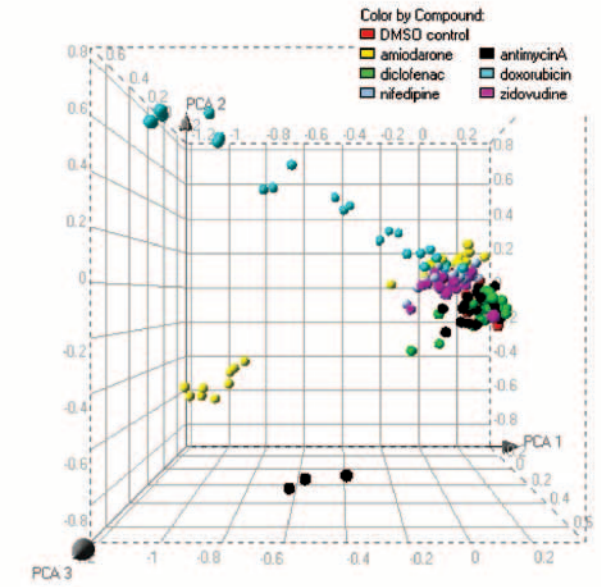


Fig 1. A multiplexed cytotoxicity assay reveals distinct differences in cell phenotype. Cytiva Cardiomyocytes were treated for 24 h with test compounds or DMSO only, and then stained for mitochondrial status (TMRM, red), calcium mobilization (Fluo-4 AM, green), DNA/nuclear status (Hoechst™ 33342, blue), and cell viability (TOTO-3 iodide, not displayed in the images). Cells were imaged live with IN Cell Analyzer 2000. Data from all six compounds were analyzed using hierarchical clustering and profiling tools accessed through the IN Cell Investigator analysis software interface; selected results are shown here.

A)



A)

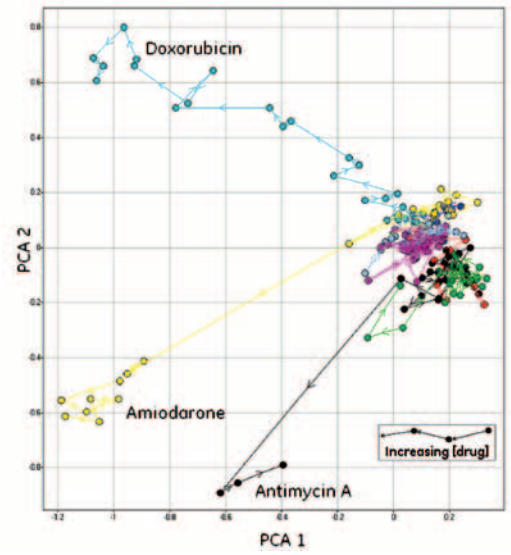


Fig 2. PCA analysis of the six-compound study reveals multiparametric differences in compound effects. **A)** Each of the 3 principle components is plotted on a separate axis; **B)** The first two principle components are plotted on the x and y axes, respectively, with arrows connecting the points to indicate increasing drug concentration. In both plots, data points are colored by compound, as shown in the key.

Heatmap visualization and multiparametric profile plots (Fig 1) revealed that amiodarone and doxorubicin elicit dramatically different multiparametric 'signatures'. These distinct signatures suggest that the two drugs can induce cell death through different signaling mechanisms, a hypothesis that is supported by published studies (3,4). It is also noteworthy that replicate samples tend to have similar signatures or profiles (i.e., they cluster together), as do different doses of the same compound. This observation is an indication that profiling on the basis of multiple parameters provides a relatively robust means of distinguishing subtle compound effects (5).

Correctly interpreting complex multiparametric data from an HCA study can be a significant challenge, especially when some of the reported measurements can be interdependent. Principle Component Analysis (PCA), accessible through the IN Cell Investigator software interface, is a powerful tool that can be used to simplify large multiparametric data sets, returning a smaller number of independent variables (principal components). In this study, 54 cellular parameters from each treatment condition were reduced to three principle components. Multidimensional plots of the principle components (Fig 2) revealed distinct clusters and dose-dependencies for the test compounds. It is readily apparent that three of the test compounds (doxorubicin, amiodarone, and antimycin A) elicit markedly different cellular phenotypes, particularly at higher doses. The phenotype induced by doxorubicin developed in a dose-dependent manner over the concentration range, while antimycin A and amiodarone elicited a threshold type of response. By contrast, diclofenac, zidovudine, and nifedipine produced PCA profiles more similar to the DMSO controls, and exhibited a smaller dynamic range of response. While these three compounds have been associated to varying extents with cardiovascular toxicity, literature suggests that their damaging effects are likely to be cumulative and less acutely cytotoxic than those of doxorubicin, amiodarone, and antimycin A.

Conclusion

Early identification of cardiotoxic compounds can be critical to reducing drug development costs and avoiding adverse reactions. High-content toxicity studies conducted with GE Healthcare's Cytiva Cardiomyocytes have the potential not only to identify compounds with potential cardiotoxic liabilities, but also to differentiate different toxic compounds by mode of action and group them based on similarities in their responses.

In this study, automated live-cell imaging and analysis yielded a wealth of detailed cellular information that was used to generate phenotypic profiles for the test compounds. Using various data clustering, profiling, and transformation tools accessible through IN Cell Investigator software, two compounds likely to mediate apoptosis by different mechanisms were readily distinguished from each other, and the dose-dependencies of their effects were examined. HCA profiling with Cytiva Cardiomyocytes provides a sensitive and robust screening approach to identify potential cardiotoxic liabilities and gain early insight into their mechanisms of toxicity.

References

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Ordering information

Product	Code number
IN Cell Analyzer 2000, with large chip CCD camera	28-9535-10
IN Cell Investigator I Seat, Web download	28-4089-71
Cytiva Cardiomyocytes (1E5), 1 × 10^5 cardiomyocytes	28-9774-35
Cytiva Cardiomyocytes (1E6), 1 × 10^6 cardiomyocytes	28-9763-98
Cytiva Cardiomyocytes (5E6), 5 × 10^6 cardiomyocytes	28-9763-99

For a detailed description of this study, visit www.gelifesciences.com and search for document 28-9859-16 under the Literature tab.