ABSTRACT

During the drug development process, late-stage detection of cardiotoxic side effects can significantly increase program costs and time to market. Failure to detect cardiotoxicity prior to launch can present a serious health risk for patients. Following costly withdrawals of a number of drugs from the market due to unexpected adverse cardiovascular effects, 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 provides differentiated cardiomyocytes derived from human embryonic stem cells. To explore the utility of GE Healthcare Cardiomyocytes in image-based assays for toxicity screening, we challenged the cells with a panel of test compounds, including those with known or suspected cardiotoxic liabilities. High-content analysis (HCA) of the results allowed us to identify cardiotoxic compounds and distinguish them from each other based on their phenotypic profiles.

RESULTS AND DISCUSSION

Dose response analysis

As the dose-response plots in Figure 4 show, doxorubicin and amiodarone toxicity were readily detected by the assay, which reported a dose-dependent decrease in mitochondrial shape (1/ form factor) and plasma membrane integrity (viability), concomitant with changes in nuclear area and intracellular calcium concentration (not shown). The toxic dose inducing half maximal plasma membrane integrity of the population (ID50) was 1.6µM and 4.5µM for doxorubicin and amiodarone respectively, on the same order of magnitude as values reported in the literature with other model systems (1). The effects of these compounds are observed for similar parameters, but the images and dose response profiles suggest different pharmacodynamics. Evidence suggests that acute doxorubicin cardiotoxicity involves cardiomyocyte apoptosis via a Bax-mediated pathway whereas the effect of amiodarone is mediated by a Bax-independent process.

METHODS

The live assay, image acquisition and analysis were performed as described in the workflow Figure 2. Cardiomyocytes were seeded onto Matrigel-coated plates following the methods for plate coating and cell preparation as described in the Cardiomyocytes User Guide (EB8908058A). After compound exposure, images of live cardiomyocytes were acquired on an IN Cell Analyzer 2000 high-content analysis system using the following excitation (λ) and emission (η) filter combinations: 350/50, 455/50 (Hoechst 33342); 490/20, 525/20 (Fluo-4 AM); 480/50, 560/20 (PI); 460/40, 530/20 (TMRM); 508/30, 550/30 (Alexa Fluor 568); 510/10, 560/30 (Alexa Fluor 633); 535/30, 570/30 (Rhodamine Red X). Total cellular and nuclear areas and natriuretic peptide levels were measured and compared to control. Data were normalized and plotted for each compound (n=3 wells per treatment condition).

As this example illustrates, cell imaging has the potential not only to identify toxic compounds with potential cardiotoxic liabilities, but also to differentiate between different toxic compounds and group them based on similarities in their responses. While high content analysis of the 4-color toxicity assay captured a wealth of cell-by-cell data from the test compounds over the dose-range tested. The higher doses. However, diclofenac, zidovudine and nifedipine have phenotypic profiles more similar to the ID50 profiles. While all of these compounds have been associated in some way with adverse cardiovascular effects, literature suggests that their damaging effects are likely to be cumulative and less acutely cytotoxic than those of doxorubicin, amiodarone and antinomy A, which trigger cell death pathways.

Automated profiling

The profiling tools used so far have enabled the key parameters important in distinguishing compound toxicity to be identified. These parameters were then used in automated profiling of the entire set of compounds at concentrations for phenotypic classification (Figure 6). The phenotypic changes induced by amiodarone, doxorubicin and antinomy A become distinguishable at certain doses and are highlighted in red. Cell phenotype is not affected by the remaining compounds over the dose-range tested.

Figure 5. Pattern detection using data clustering and profiling tools. a) An example high-content dataset consists of data from the top 4 doses for 6 compounds on the same 4 color channel. b) Multi-parametric phenotypic profiles for the 6 compounds were plotted for each dose using a parallel axis profile plot (Figure 5B), each vertical line in a given profile represents a compound. The profiles for these two compounds show some degree of separation, but the high throughputs automated imaging with IN Cell Analyzer 2000 and high content analysis of the resulting multi-parametric phenotypic signatures.