High Content Screening Analysis of Phospholipidosis

Phospholipidosis, a lipid storage disorder characterized by excessive accumulation of phospholipids in lysosomes, is an adverse side effect of drug exposure. During development, candidate drugs are analyzed for their potential to induce phospholipidosis. Analysis is currently performed using electron microscopy on tissue samples, but this is expensive, time consuming and performed at a late stage in drug development.

In this study, researchers have validated an in vitro high content screening assay for phospholipidosis detection using the Operetta® High Content Imaging System with Harmony® High Content Imaging and Analysis Software. CHO-K1 and HepG2 cells were incubated with different concentrations of 56 test compounds and with the fluorescent phospholipid biomarker NBD-PE. Cells were counterstained with Hoechst 33342 and DRAQ5™ and images were acquired using the Operetta system.

Automated image analysis and quantification of compound-induced NBD-PE accumulation was performed using Harmony software. As a first step, the nuclei and cytoplasmic outlines of each cell were detected (Figure 1). Accumulation of NBD-PE in the cytoplasmic area of each cell was then quantified by multiplying the mean NBD-PE fluorescence intensity of positive cells with the number of positive cells identified per well by scoring the fluorescence intensity level against the overall background level. The localization of NBD-PE in lysosomes was confirmed via colocalization experiments performed using the Operetta system with NBD-PE and a fluorescent lysosomal marker.

Figure 2 shows the quantified amounts of NBD-PE accumulation for each tested dose of a negative and positive compound. In both cell lines, exposure to the negative control did not induce NBD-PE accumulation, whereas exposure to the positive control induced a clear dose-dependent accumulation of NBD-PE.

The high content in vitro assay described here has a high sensitivity of 92% and 88% (n=25) and high specificity of 87.1% and 80.6% (n=31) for predicting phospholipidosis for CHO-K1 and HepG2 cells respectively, and will therefore be a useful tool for the early selection of drug candidates.

**Figure 1:** Automated detection of nuclei and cytoplasm (following Hoechst 33342 and DRAQ5™ staining respectively) and NBD-PE accumulation in the cytoplasm of CHO-K1 cells. Here, cells were treated with 3.16 µM of the phospholipidosis inducer, amiodarone. Following amiodarone treatment, the NBD-PE fluorescence in the cytoplasm was quantified using Harmony Software.

**Figure 2:** Quantification of the effects of a negative compound (sulindac) and a phospholipidosis inducer (amitryptiline) on NBD-PE accumulation. Cells were incubated with increasing concentrations of sulindac and amitryptiline. NBD-PE accumulation (CHO-K1 = gray bars, HepG2 = black bars) is expressed as a % of the effect of the phospholipidosis inducer amiodarone at 3.16 µM (CHO-K1) or 31.6 µM (HepG2) set at 100%. (*) Indicates a significant increase in NBD-PE accumulation (P < 0.025). Cytotoxicity (CHO-K1 = gray lines, HepG2 = black lines) is shown as a % decrease in the number of cells compared with the control.