Progress Toward a High-Throughput Assay for a hERG Channel Blocker

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Over the last few years, many non-cardiovascular drugs representing a wide variety of structural classes have come under scrutiny or been withdrawn from the market because of reported incidences of ventricular arrhythmias, particularly torsade de pointes (TdP). The mechanism by which these drugs cause TdP is believed to be through blockade of the human ether-a-go-go-related (hERG) ion channel. The hERG channel is a voltage-activated, inwardly-rectifying potassium channel that is a major contributor to the repolarization of ventricular action potentials. Blockade of hERG increases the duration of cardiac action potentials, which leads to prolonged ventricular depolarization and long QT interval on the ECG.

Because of the potentially fatal nature of compounds' interaction with hERG, it is prudent to include a test for hERG blockade as a component of any small molecule project's battery of secondary assays. Whole-cell voltage-clamp is the most accurate and reliable method for detecting potassium channel blockers, but an efficient, predictive high-throughput hERG assay is needed for eliminating compounds from a list of hits before moving on to time-consuming electrophysiology assays.

Here, progress toward a semi-automated assay for detection of hERG blockers is presented. Our assay uses a membrane potential-sensitive dye on Molecular Devices' Fluorometric Imaging Plate Reader (FLIPR) to detect depolarization of CHO cells stably expressing hERG. The assay uses a double-addition protocol. Baseline fluorescence is measured in low K+ buffer. Then fluorescence is measured after incubation in compound and then again after addition of high K^+. The process is duplicated in non-transfected parental CHO cells. Thus, each compound is profiled based on its effect in cells in low K^+ and on the cells' response to high K^+. These compound profiles are compared to electrophysiology data for a variety of compounds in order to validate the assay.