False-positive and -negative effects in in vitro assays for predicting QT prolongation effects of drugs

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1. Introduction

Two kinds of in vitro assay, the APD (action potential duration) assay, which measures action potentials in cardiac muscles using the microelectrode technique, and the hERG (human ether-a-go-go-related gene) assay, which measures the hERG current in H9c2 transfected cell lines using the patch-clamp technique, are widely used in safety pharmacology studies to predict effects of drugs on QT interval prolongation in the electrocardiogram. However it has become clear that there are some drugs that yield false positives or false negatives in both assays. It is very important to understand the mechanism by which this happens.

In our research, we used drugs that have already been reported as having a prolongation effect on the QT interval, but yield negative results in the APD or hERG assays, to investigate the possibility of improving the accuracy of both assays. We also conducted the same kind of investigation using drugs that have no prolongation effect on the QT interval, but yield positive results in one of the assays.

2. Test substances

E-4031

Terfenadine

Verapamil

dl-Sotalol

3. Methods

Test methods employed to determine the suppressive effects of test substances on the hERG potassium channel transfected in HEK293 cells and action potentials in isolated guinea pig papillary muscles. A: A schematic diagram of the test substance application system for hERG assay. B: The voltage clamp protocol and currents recorded from a hERG-transfected cell. The cell was held at -70 mV and depolarized to 0 mV for 0.75 sec to activate and slightly inactivate the hERG potassium channels, and then repolarized to -50 mV for 10 sec to induce the tail current. E-4031 at 100 nmol/L, was applied to the cell for 10 min. The temperature of extra cellular solution was maintained at 37±1°C. C: A schematic diagram of the test substance application system for action potential duration assay. D: Action potentials recorded from an isolated guinea pig papillary muscle. Electric stimul (Voltage: Two times higher than the threshold, Pulse length: 1 msec) were delivered to the sample muscle at a frequency of 1 Hz using a stimulator and isolator. E-4031 was applied cumulatively from lower concentration to the sample muscle for 10 min at each concentration. The temperature was maintained at 37±1°C.

4. Effects of test substances on the hERG channel

5. Effects of test substances on action potential duration

6. Effects of test substances on action potential parameters

7. Summary and Conclusion

Table 2. Effects of E-4031, terfenadine, verapamil and dl-sotalol on action potential durations and hERG current.

Test substance | APD assay | EC50 [APD] (mM) | EC50 [Transliteration] (mM) | hERG assay | IC50 value (mM) |
--- | --- | --- | --- | --- | --- |
E-4031 | 19 | 9 | 20 | 100 nmol/L |
Terfenadine | - | 6500 | 14 | |
Verapamil | - | 230 | |
dl-Sotalol | 8000 | 3100 | 12000 |

E-4031 showed concentration-dependent effect on action potentials as well as the hERG current. Terfenadine did not show significant effects on action potential durations up to 10 μmol/L. In contrast, the compound remarkably suppressed the hERG current with an IC50 value of 14 nmol/L. Verapamil showed concentration-dependent suppression effect on the hERG current with an IC50 value of 230 nmol/L. However, the compound did not suppress the hERG current at concentrations where the action potential durations were significantly extended. dl-Sotalol showed concentration-dependent effect on action potentials as well as the hERG current. However, the compound did not suppress the hERG current at concentrations where the action potential durations were significantly extended. There are compounds that show potent hERG current inhibition, but do not extend action potential durations in cardiac muscles. The integrated assessment are needed to predict effects of drugs on QT interval prolongation.