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

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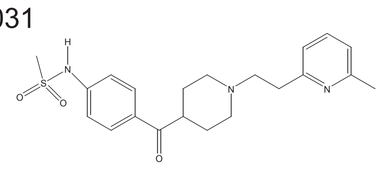
1. Introduction

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 hERG 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.

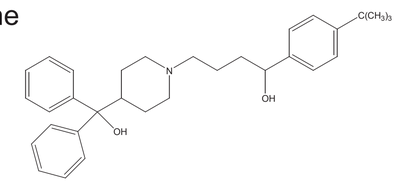
In our research, we used drugs that have already been reported as having a prolongation effect on the QT interval, but yield negative results in the APD or hERG assays, to investigate the possibility of improving the accuracy of both assays. We also conducted the same kind of investigation using drugs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

2. Test substances

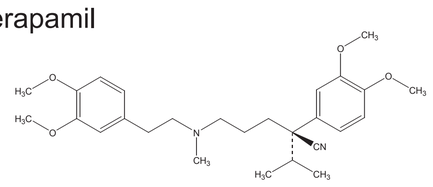
E-4031



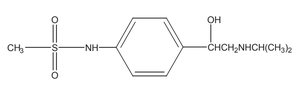
Terfenadine



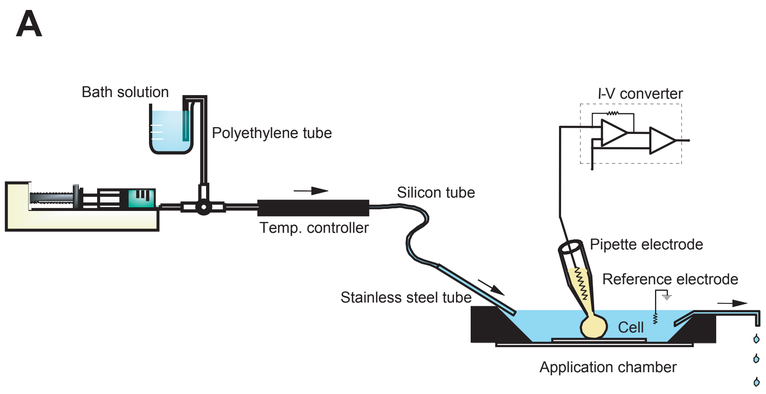
Verapamil



dl-Sotalol

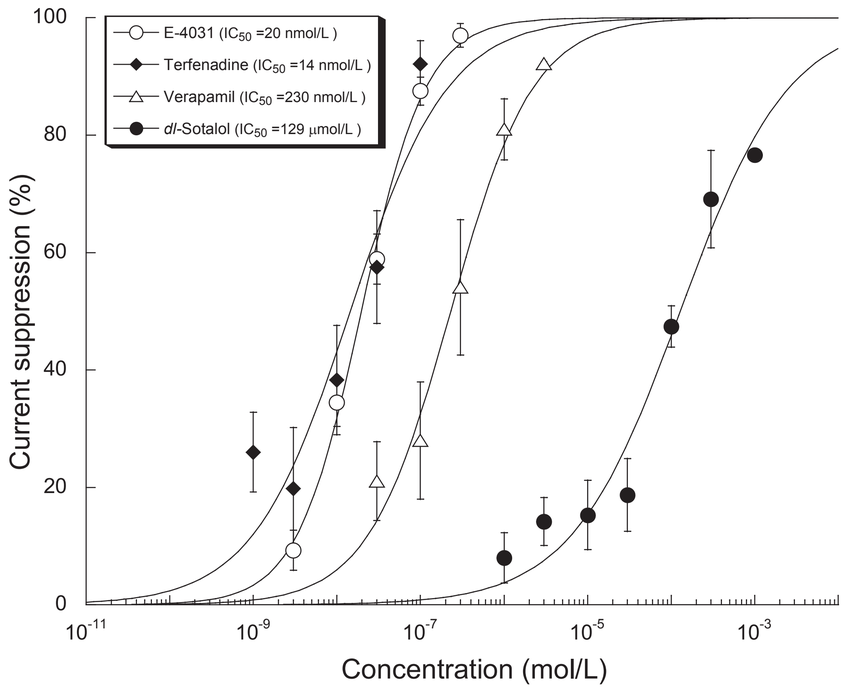


3. Methods



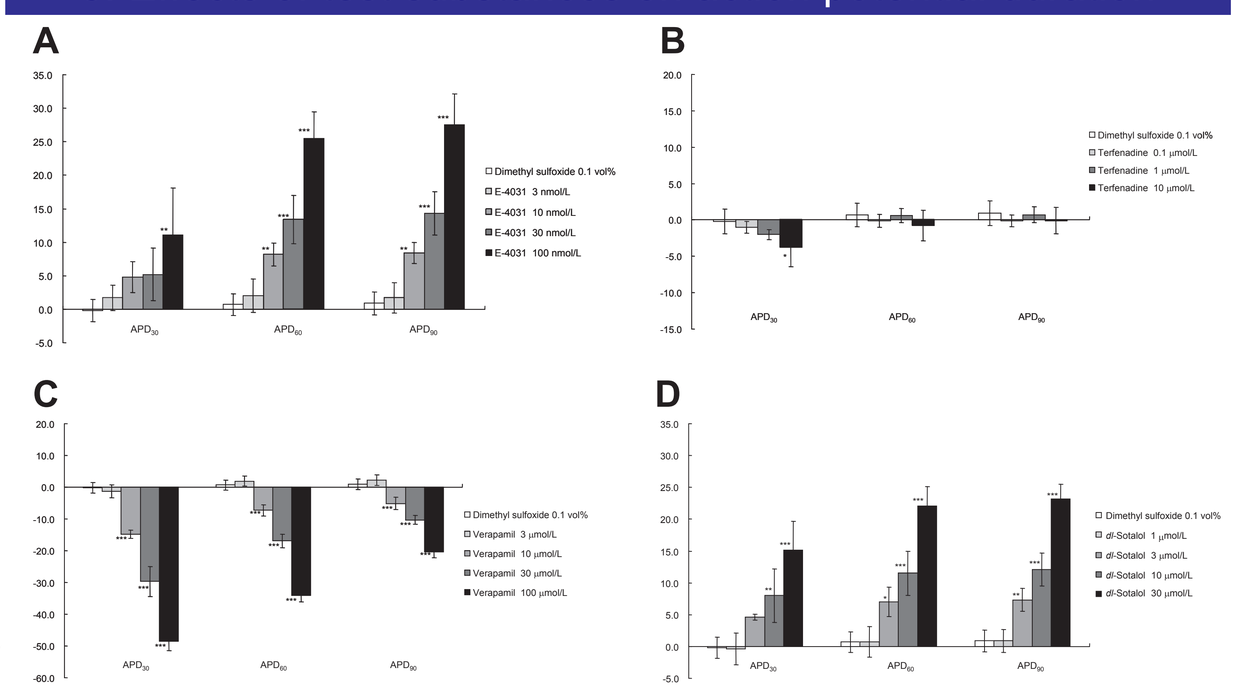
Test methods employed to determine the suppressive effects of test substances on the hERG potassium channel transfected in HEK293 cells and action potentials in isolated guinea pig papillary muscles. A; A schematic diagram of the test substance application system for hERG assay. B; The voltage clamp 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 0.75 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; 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 stimuli (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℃.

4. Effects of test substances on the hERG channel



Concentration-response relationship of test substances on hERG potassium channel currents in HEK293 cells. The value of the current used to determine the effects of a test substance is the peak outward tail current amplitude repolarized at -50 mV following the initial depolarizing step to 0 mV from a holding potential of -70 mV. These pulses were given every 15 sec. The percent suppression of the hERG current is plotted as a function of the test substance concentration. Data are fitted using the Hill equation. Each point indicates the mean ± S.D. (n=3 or 4).

5. Effects of test substances on action potential duration



Effects of E-4031, terfenadine, verapamil and *dl*-sotalol on action potential durations in isolated guinea pig ventricular papillary muscles. Each column represents the mean ± S.D. (n=4). *, ** and ***: Significant differences at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, between dimethyl sulfoxide and each test substance by Dunnett's multiple comparison test.

6. Effects of test substances on action potential parameters

Table 1. Effects of E-4031, terfenadine, verapamil and *dl*-sotalol on action potentials in isolated guinea pig ventricular papillary muscles

Test substance	Concentration	RMP (%⊿)	APA (%⊿)	dV/dt max (%⊿)	APD_{30} (%⊿)	APD_{60} (%⊿)	APD_{90} (%⊿)	APD_{30-90} (%⊿)	APD_{30-60} (%⊿)	APD_{60-90} (%⊿)
Dimethyl sulfoxide	0.1 vol%	-0.1 ± 0.3 ± 0.1	0.5 ± 0.5 ± 0.2	3.3 ± 10.0 ± 5.0	-0.2 ± 1.7 ± 0.8	0.7 ± 1.6 ± 0.8	0.9 ± 1.7 ± 0.8	3.3 ± 5.0 ± 2.5	3.8 ± 5.5 ± 2.7	2.1 ± 5.0 ± 2.5
E-4031	3 nmol/L	-0.3 ± 0.6 ± 0.3	-0.3 ± 1.2 ± 0.6	5.4 ± 9.0 ± 4.5	1.7 ± 1.9 ± 1.0	2.0 ± 2.5 ± 1.2	1.7 ± 2.3 ± 1.1	2.0 ± 3.2 ± 1.6	3.2 ± 4.8 ± 2.4	-0.8 ± 2.8 ± 1.4
E-4031	10 nmol/L	-0.2 ± 0.8 ± 0.4	-0.3 ± 1.3 ± 0.7	7.4 ± 16.5 ± 8.3	4.8 ± 2.3 ± 1.2	8.2 ± 1.7 ** ± 0.8	8.4 ± 1.6 ** ± 0.8	16.5 ± 2.0 ** ± 1.0	19.7 ± 5.8 ± 2.9	9.1 ± 6.2 ± 3.1
E-4031	30 nmol/L	-1.1 ± 0.9 ± 0.5	-0.9 ± 1.5 ± 0.7	11.8 ± 15.8 ± 7.9	5.2 ± 3.9 ± 1.9	13.4 ± 3.6 *** ± 1.8	14.3 ± 3.2 *** ± 1.6	34.8 ± 5.5 *** ± 2.8	40.7 ± 11.5 *** ± 5.8	20.7 ± 10.2 ± 5.1
E-4031	100 nmol/L	-0.8 ± 0.8 ± 0.4	-0.8 ± 1.4 ± 0.7	15.6 ± 18.6 ± 9.3	11.1 ± 7.0 ** ± 3.5	25.5 ± 4.0 *** ± 2.0	27.5 ± 4.6 *** ± 2.3	63.9 ± 5.7 *** ± 2.9	72.9 ± 14.6 *** ± 7.3	41.9 ± 17.5 *** ± 8.8
Terfenadine	0.1 μmol/L	-0.3 ± 0.1 ± 0.1	-0.3 ± 0.3 ± 0.2	1.2 ± 2.1 ± 1.1	-1.0 ± 0.8 ± 0.4	-0.1 ± 0.9 ± 0.4	-0.1 ± 0.8 ± 0.4	2.2 ± 1.0 ± 0.5	3.5 ± 1.6 ± 0.8	-0.7 ± 0.8 ± 0.4
Terfenadine	1 μmol/L	-0.4 ± 0.4 ± 0.2	-0.4 ± 0.5 ± 0.3	-2.2 ± 2.3 ± 1.2	-2.0 ± 0.7 ± 0.4	0.6 ± 1.0 ± 0.5	0.7 ± 1.1 ± 0.5	7.3 ± 2.9 ± 1.4	9.4 ± 2.6 ± 1.3	2.4 ± 3.8 ± 1.9
Terfenadine	10 μmol/L	-0.1 ± 0.4 ± 0.2	-1.2 ± 0.9 * ± 0.5	-4.5 ± 9.8 ± 4.9	-3.8 ± 2.6 * ± 1.3	-0.8 ± 2.1 ± 1.1	-0.1 ± 1.8 ± 0.9	8.8 ± 0.9 ± 0.5	10.0 ± 3.0 ± 1.5	6.4 ± 4.2 ± 2.1
Verapamil	3 μmol/L	-0.3 ± 0.5 ± 0.3	-1.4 ± 0.6 ± 0.3	-2.1 ± 2.5 ± 1.2	-1.3 ± 2.1 ± 1.0	1.9 ± 1.6 ± 0.8	2.2 ± 1.6 ± 0.8	9.3 ± 2.4 ± 1.2	11.6 ± 2.1 ± 1.0	4.3 ± 3.5 ± 1.8
Verapamil	10 μmol/L	-0.5 ± 0.5 ± 0.2	-2.3 ± 0.9 ± 0.4	-7.0 ± 8.4 ± 4.2	-14.9 ± 1.3 *** ± 0.6	-7.3 ± 1.7 *** ± 0.8	-5.1 ± 1.9 *** ± 1.0	14.6 ± 8.7 ± 4.3	15.3 ± 10.0 ± 5.0	13.1 ± 6.0 ± 3.0
Verapamil	30 μmol/L	-0.3 ± 0.8 ± 0.4	-3.4 ± 1.2 * ± 0.6	-10.5 ± 14.5 ± 7.2	-29.7 ± 4.7 *** ± 2.4	-16.9 ± 2.1 *** ± 1.1	-10.3 ± 1.4 *** ± 0.7	29.2 ± 9.9 *** ± 5.0	21.2 ± 9.9 * ± 4.9	46.0 ± 13.9 *** ± 6.9
Verapamil	100 μmol/L	-1.0 ± 1.0 ± 0.5	-8.3 ± 3.1 *** ± 1.6	-22.1 ± 13.2 * ± 6.6	-48.6 ± 2.9 *** ± 1.4	-34.1 ± 2.1 *** ± 1.0	-20.4 ± 1.9 *** ± 1.0	36.8 ± 7.7 *** ± 3.8	8.9 ± 5.4 ± 2.7	96.5 ± 18.1 *** ± 9.1
dl-Sotalol	1 μmol/L	-0.1 ± 0.7 ± 0.4	0.2 ± 0.4 ± 0.2	3.9 ± 4.1 ± 2.0	-0.4 ± 2.5 ± 1.3	0.7 ± 2.4 ± 1.2	0.9 ± 1.8 ± 0.9	3.7 ± 2.0 ± 1.0	4.4 ± 2.9 ± 1.5	2.1 ± 4.0 ± 2.0
dl-Sotalol	3 μmol/L	-0.1 ± 0.2 ± 0.1	0.3 ± 0.4 ± 0.2	3.2 ± 6.1 ± 3.0	4.6 ± 0.5 ± 0.3	7.0 ± 2.3 * ± 1.1	7.3 ± 1.8 ** ± 0.9	13.0 ± 4.7 * ± 2.3	14.7 ± 7.9 ± 3.9	9.3 ± 6.2 ± 3.1
dl-Sotalol	10 μmol/L	-0.4 ± 0.5 ± 0.2	0.2 ± 0.7 ± 0.3	0.2 ± 6.3 ± 3.2	8.0 ± 4.2 ** ± 2.1	11.5 ± 3.5 *** ± 1.7	12.1 ± 2.6 *** ± 1.3	21.0 ± 4.4 *** ± 2.2	22.7 ± 8.2 ** ± 4.1	17.1 ± 7.3 * ± 3.7
dl-Sotalol	30 μmol/L	-0.3 ± 0.6 ± 0.3	-0.5 ± 1.0 ± 0.5	-9.4 ± 3.1 * ± 1.6	15.1 ± 4.6 *** ± 2.3	22.1 ± 3.0 *** ± 1.5	23.2 ± 2.3 *** ± 1.1	40.7 ± 2.7 *** ± 1.3	44.5 ± 10.1 *** ± 5.1	32.6 ± 11.6 *** ± 5.8

APD_{30}, APD_{60} and APD_{90} represent time periods for 30%, 60% and 90% repolarization of action potential, respectively; RMP, resting membrane potential; APA, action potential amplitude; dV/dt max, maximum rate of rise of action potential; APD_{30-90}, difference between APD_{90} and APD_{30}; APD_{30-60}, difference between APD_{60} and APD_{30}; APD_{60-90}, differences between APD_{90} and APD_{60}. Values represent the mean ± S.D. (upper, n=4) and ± S.E.M. (lower, n=4). *, ** and ***: Significant differences at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, between dimethyl sulfoxide and each test substance by Dunnett's multiple comparison test.

7. Summary and Conclusion

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

Test substance	APD assay EC_{10} [APD_{90}] (nmol/L)	APD assay EC_{10} [Triangulation] (nmol/L)	hERG assay IC_{50} value (nmol/L)
E-4031	19	9	20
Terfenadine	-	6500	14
Verapamil	-	-	230
dl-Sotalol	8000	3100	129000

- 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 IC_{50} value of 14 nmol/L.
- Verapamil showed concentration-dependent suppression effect on the hERG current with an IC_{50} value of 230 nmol/L. However, the compound shortened the action potential durations in a concentration-dependent manner between 10 and 100 μmol/L.
- *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.