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Original article

Automated electrophysiology in the preclinical evaluation of drugs for potential QT prolongation

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Abstract

Introduction: The human ether-a-go-go-related gene (hERG) potassium channel plays a major role in the electrical conductances involved in human heart repolarization. Drugs that decrease hERG K^+ currents are at risk to produce a prolongation of the cardiac action potential, resulting in an increase of the QT interval. Drug-induced QT prolongation or acquired long QT (aLQT) can lead to a fatal arrhythmia known as *Torsade de Pointes* (TdP). Electrophysiological methods are the best approach to evaluate potential drug candidates for hERG current inhibition. Here we identify limitations with the PatchXpress 7000A automated electrophysiology instrument and describe hERG protocol optimizations necessary for reliable preclinical assessment. **Methods:** The PatchXpress 7000A automated electrophysiology system was used to evaluate a group of drugs with known hERG activity under voltage clamp conditions. We used a recombinant cell line expressing hERG, and assessed the inhibition of hERG K^+ currents at different drug concentrations. These data were used to determine hERG IC_{50} values and compare assay parameters under different recording conditions. **Results:** We found that due to limitations with the PatchXpress 7000A instrument, repeated compound additions were critical for achieving steady state drug concentrations that generated data comparable to standard patch clamp methods, particularly when similar voltage pulse protocols were implemented. Some discrepancies were observed between the PatchXpress 7000A and standard patch clamp techniques including shifts in IC_{50} values for very hydrophobic compounds. Most hERG IC_{50} values were within 3-fold of standard patch clamp IC_{50} values. **Discussion:** Automation of electrophysiology technologies has greatly improved the throughput of assessing lead drug candidates for hERG liability. To maintain hERG data quality comparable to standard patch clamp techniques, the PatchXpress 7000A instrument limitations should be recognized and protocols optimized accordingly to ensure accuracy.

Keywords: Cardiac safety; CHO cells; hERG; Methods; PatchXpress 7000A; QT prolongation

1. Introduction

The human ether-a-go-go-related gene (hERG) potassium channel underlies I_{Kr}, a major repolarizing current in the human heart. Mutations in hERG can lead to a prolongation in the electrocardiogram QT interval known as long QT (LQT) and certain drugs can decrease I_{Kr}, a condition known as acquired long QT (aLQT). Prolongation in the QT interval can result in delayed ventricular repolarization and in some cases, *Torsade de Pointes* (TdP), a potentially fatal arrhythmia (Chiang & Roden, 2000). A number of marketed drugs have been withdrawn due to drug-induced QT prolongation (Woolsey, 2005). Although the precise mechanisms and factors that lead to aLQT induced arrhythmias are relatively unknown, it is without question the block of the hERG ion channel and corresponding decrease in I_{Kr} are an underlying cause of aLQT (Curran et al., 1995; Roy, Dumaine, & Brown, 1996; Salata et al., 1995; Sanguinetti, Jiang, Curran, & Keating, 1995). The ability to rank order compounds relative to their therapeutic potencies is critical for establishing a therapeutic index (Guth, Germeyer, Kolb, & Markert, 2004; Roden, 2004).

The ICH S7B guidelines are still in draft form; the specific recommendations regarding hERG assays are

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vague. Several hERG assay parameters can introduce variability within the results, including solution composition, temperature and voltage step protocol (Kirsch et al., 2004; Wang et al., 2003; Zhang, Kehl, & Fedida, 2003; Zhou et al., 1998). The results of hERG assays are used to evaluate a potential drug candidate in combination with other assays as an integrative approach for cardiovascular safety risk assessment. Typically, this has been done through safety margins using a hERG IC_{50} (concentration of compound resulting in 50% inhibition of hERG current) in proportion to the concentration of free compound in plasma (free C_{max}). It has been suggested that a ratio of 30-fold significantly decreases the risk of QT prolongation (Redfern et al., 2003).

hERG IC_{50} values are critical measurements and should be reported using the most sensitive and accurate methods available. With the advent of automated electrophysiology (Bennett & Guthrie, 2003; Shieh, 2004; Wood, Williams, & Waldron, 2004), new challenges have arisen which need to be addressed in order to assure quality in data output. Here, we discuss protocol optimization required when using the PatchXpress 7000A automated electrophysiology system in order to match historical data generated from standard patch clamp techniques. Our findings suggest: 1) When testing very potent compounds (standard patch clamp IC_{50} <100 nM), and highly hydrophobic compounds (calculated logarithmic P octanol/water measurement (cLogP)>5), the IC_{50} values from concentration–response curves tend to be right-shifted (less potent). This appears to be largely due to the limitation of compound delivery method and possibly compound non-specific binding to the surfaces exposed. 2) A lengthened voltage step protocol shifts IC_{50} values towards standard patch clamp values for most of the compounds tested. However, if multiple compound additions were employed to achieve appropriate steady state compound concentrations, the lengthened protocol was generally not required to produce an accurate IC_{50} value. This was most obvious for compounds with larger IC_{50} values ($\geq$100 nM) and less hydrophobic compounds. We find that the PatchXpress 7000A performance was best when applying the same protocol used for standard patch clamp methods, with up to six additions for each compound concentration tested (1 min apart). The implementation of these assay parameters enabled higher throughput patch clamp with the PatchXpress 7000A while maintaining data quality.

2. Materials and methods

2.1. Cell culture

Chinese hamster ovary (CHO) cells stably expressing the human ether-a-go-go-related gene (hERG) was generated by Roche, Nutley (Guthrie, Livingston, Gubler, & Garippa, submitted for publication). Cell culture media components included Ex-cell 301 (JRH, Cat JRH-14331), 10% Fetal Bovine Serum (Gibco, Cat 16140-089) and 0.25 mg/ml Geneticin (Gibco, Cat 10131-035). Cells were grown at 37±2 °C, supplemented with 5% CO_2.

2.2. Compounds

Dofetilide, sibutramine and Roche internal compounds (RO) were synthesized by Roche. Cisapride was obtained from Research Diagnostics Inc. (Cat 81098-60-4). Olanzapine was purchased from Toronto Research Chemicals (Cat 0253750). All other compounds were purchased from Sigma. Positive control used was E-4031 (Sigma, Cat M-5060). Compound vehicle used was dimethyl sulfoxide (DMSO, J.T. Baker, Cat 9224-01). Final DMSO concentrations in testing solutions were $\leq$0.3%. For both PatchXpress 7000A and standard patch clamp, control current was determined using External Buffer containing the same concentration of DMSO as that in compound testing solution. Compound stock solutions were prepared in glass vials (Wheaton, Cat 224982), and diluted to 50 ml polypropylene conical tube (Becton Dickinson Labware, Falcon 2098) with External Buffer for standard patch clamp setup. Compound testing solutions used for PatchXpress 7000A experiments were prepared in glass vials and loaded into glass coated 96 well microplates (Sun-Sri, Cat 400 042). All compound testing solutions were used within 12 h. Stock solutions were stored for short term at 4±2 °C and long term at −80±5 °C. Calculated logarithmic P octanol/water measurements (cLogP) were determined from http://www.daylight.com/ (Leo et al., 1975).

2.3. Electrophysiology

2.3.1. Solutions

Electrophysiology recording solutions for both standard and automated patch clamp include Internal Buffer (in mM, from Sigma unless otherwise noted): 140 KCl (Cat P-9541), 6 EGTA (Cat E-3889), 5 Hepes (Cat H-3784), 5 $MgCl_2$ (Cat M-1028), 5 ATP-Na_2 (Cat A-2383) pH 7.2 with KOH (J.T. Baker, Cat 3143-01); External Buffer (in mM, from Sigma unless otherwise noted): 150 NaCl (Cat S-3014), 10 Hepes (Cat H-3784), 4 KCl (Cat P-9541), 1.2 $CaCl_2$ (Cat C-3306), 1 $MgCl_2$ (Cat M-1028), pH to 7.4 with HCL (J.T. Baker Cat 5619-02).

2.4. Standard patch clamp

CHO-hERG cells were visualized using a Nikon TE2000-S inverted microscope. Borosilicate glass pipettes were pulled (Sutter Instruments Micropipette Puller P-97) to generate a tip resistance of approximately 1–2 MΩ. Voltage clamp was carried out with the MultiClamp 700A-2 amplifier and data collected with the Digidata Acquisition System (1322A, Molecular Devices Corporation). Data was analyzed using pCLAMP 9.0 software (Molecular Devices