British Journal of Pharmacology (2006) 149, 457–459

0007–1188/06 $30.00
www.brjpharmacol.org

COMMENTARY

Combined hERG channel inhibition and disruption of trafficking in drug-induced long QT syndrome by fluoxetine: a case-study in cardiac safety pharmacology

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Drug-induced prolongation of the rate-corrected QT interval (QT_CI) on the electrocardiogram occurs as an unwanted effect of diverse clinical and investigational drugs and carries a risk of potentially fatal cardiac arrhythmias. *hERG* (human *ether-à-go-go-related gene*) is the gene encoding the α-subunit of channels mediating the rapid delayed rectifier K^+ current, which plays a vital role in repolarising the ventricles of the heart. Most QT_CI prolonging drugs can inhibit the function of recombinant hERG K^+ channels, consequently *in vitro* hERG assays are used widely as front-line screens in cardiac safety-testing of novel chemical entities. In this issue, Rajamani and colleagues report a case of QT_CI prolongation with the antidepressant fluoxetine and correlate this with a dual effect of the drug and of its major metabolite norfluoxetine on hERG channels. Both compounds were found to produce an acute inhibition of the hERG channel by pharmacological blockade, but in addition they also were able to disrupt the normal trafficking of hERG protein to the cell membrane. Mutations to a key component of the drug binding site in the S6 region of the channel greatly attenuated channel block, but did not impair disruption of trafficking; this suggests that channel block and drug effects on trafficking were mediated by different mechanisms. These findings add to growing evidence for disruption of hERG channel trafficking as a mechanism for drug-induced long QT syndrome and raise questions as to possible limitations of acute screening methods in the assessment of QT_cI prolonging liability of drugs in development.
British Journal of Pharmacology (2006) **149,** 457–459. doi:10.1038/sj.bjp.0706890; published online 11 September 2006

Keywords: arrhythmia; fluoxetine; hERG; Human *ether-à-go-go-related gene*; long QT syndrome; norfluoxetine; potassium channel; Prozac; QT interval; safety pharmacology

Abbreviations: hERG, human *ether-à-go-go-related gene*; I_{hERG}, current carried by recombinant hERG channels; I_{Kr}, current of the rapid delayed rectifier K^+ channel; NCE, novel chemical entity; QT_CI, rate-corrected QT interval

The QT interval on the body surface electrocardiogram represents the time interval between initiation of ventricular depolarization and completion of ventricular repolarization. Prolonged QT intervals typically result from delayed repolarization of cardiac ventricular action potentials and are associated with an increased risk of the potentially fatal polymorphic ventricular tachycardia, torsades de pointes (TdP; Yap and Camm, 2003). QT interval prolongation can result from gene mutations or, more frequently, arises as an unwanted side effect of clinically used drugs. Rate-corrected QT intervals (QT_CI) for males and females of >440 and >460 ms, respectively, are considered to be prolonged, with arrhythmias most commonly associated with QT_CI values of $\geqslant 500$ ms (Yap and Camm, 2003). The drugs that exhibit a propensity to prolong the QT_CI are both structurally and therapeutically diverse (e.g. Witchel and Hancox, 2000; Yap and Camm, 2003, and also see www.torsades.org). However, the majority of such drugs have been observed to exert a common effect: pharmacological inhibition of the 'rapid delayed rectifier' potassium channel current (I_{Kr}), which is vital for normal ventricular repolarization (Witchel and Hancox, 2000; Sanguinetti and Mitcheson, 2005; Sanguinetti and Tristani-Firouzi, 2006). The human *ether-à-go-go-related gene* (*hERG*) encodes functional I_{Kr} channels and recombinant hERG channels are able to interact with diverse drugs owing to a combination of a large inner cavity and involvement in the drug binding site of aromatic amino-acid residues in the S6 helices of the channel (Sanguinetti and Mitcheson, 2005; Sanguinetti and Tristani-Firouzi, 2006). Although drug-induced torsadogenesis is complex

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Received 24 July 2006; accepted 31 July 2006; published online 11 September 2006

and an area of active debate (e.g. Hoffmann and Warner, 2006), owing to the strong association between QT_CI prolongation and hERG channel blockade, hERG channel inhibition offers a convenient surrogate marker for drug-induced QT_cI prolongation. Recombinant hERG channel assays therefore constitute an important early screen for testing novel chemical entities (NCEs) for their QT_cI-prolonging liability. Moreover, it is hoped that a detailed understanding of the molecular determinants of hERG channel blockade, together with hERG pharmacophore models may ultimately help design out hERG blockade *in silico*, thereby minimizing attrition of drugs in the design and development process (Recanatini *et al*., 2005; Sanguinetti and Mitcheson, 2005). An article in this issue of the *BJP*, dealing with QT_cI prolongation and hERG inhibition by the serotonin-selective-reuptake inhibitor (SSRI) antidepressant, fluoxetine (Prozac) provides pause for thought in this regard (Rajamani *et al*., 2006). These authors provide evidence for a dual mechanism for attenuated hERG channel function by fluoxetine that incorporates both pharmacological blockade and a disruption of the normal trafficking of the hERG channel protein to the cell membrane.

Although SSRIs are considered to be relatively safe in overdose (Isbister *et al*., 2004), fluoxetine is occasionally associated with QT_CI prolongation, with published cases in the last 2 years including an elderly individual (Wilting *et al*., 2006), an individual taking other QT_CI-prolonging drugs (Nykamp *et al*., 2005), and an infant born to a mother taking fluoxetine (Dubnov *et al*., 2005). In the present study of Rajamani and co-workers, an adult patient had a markedly prolonged QT_CI of 625 ms, following intentional fluoxetine overdose. The authors hypothesized that this resulted from a reduction in hERG current (I_{hERG}), which is concordant with a susceptibility of the hERG channel to pharmacological blockade by fluoxetine that had been reported previously (Thomas *et al*., 2002; Witchel *et al*., 2002). However, no such data were available before this study on the major, long-lasting metabolite of fluoxetine -norfluoxetine. Using a combination of conventional whole-cell voltage-clamp and ventricular action potential (AP) clamp, Rajamani and co-workers provide convincing evidence that fluoxetine and norfluoxetine are both effective as I_{hERG} inhibitors, although with fluoxetine slightly the more potent of the two compounds. Thus, levels of both the parent compound and of its major metabolite are likely to be relevant to potential I_{Kr}/hERG blockade *in vivo* and, by extension, to possible QT_CI prolongation.

It may seem curious, therefore, that both fluoxetine and norfluoxetine have been reported to produce action potential shortening rather than lengthening, when applied acutely to isolated cardiac myocytes at concentrations relevant to those reported here to block I_{hERG} (Pacher *et al*., 2000; Magyar *et al*., 2004). The study by Rajamani and co-workers demonstrates that neither fluoxetine nor norfluoxetine suppressed sodium currents carried by heterologously expressed SCN5A Na channels, although native cardiac ion channel currents were not investigated. However, both fluoxetine and norfluoxetine have been previously shown to suppress native cardiac L-type calcium current (Pacher *et al*., 2000; Magyar *et al*., 2004), which is a key player in maintaining the ventricular action potential plateau. Thus, the effects on native cardiac tissue of acutely applied fluoxetine and norfluoxetine are unlikely to be limited to a single ion channel type.

What then of the effects of the compounds over a longer time-scale? Macroscopic currents elicited during a cardiac action potential reflect the biophysical properties of the channels and the number of functional channels expressed on the surface membrane. Channel trafficking is integral to functional hERG channel biogenesis and surface expression. Its disruption leads to a reduction in number of functional channels at the cell membrane and is an important mechanism by which a number of hERG mutants cause the LQT2 form of congenital long QT syndrome (Delisle *et al*., 2004). Recently, reports have emerged of disruption of hERG trafficking by some agents associated with acquired QT_CI prolongation (Ficker *et al*., 2004; Cordes *et al*., 2005; Kuryshev *et al*., 2005; Wible *et al*., 2005). In the present study, Rajamani *et al*. (2006) have used Western blotting to demonstrate a decrease in mature hERG protein by prolonged (24 h) exposure to either fluoxetine and norfluoxetine, with similar concentration-dependent profiles to those seen for acute I_{hERG} blockade. The potent hERG blockers cisapride and E-4031 were used as comparators and had no discernible effect on hERG trafficking at concentrations vastly exceeding those that block I_{hERG}. Data from laser scanning confocal microscopy were not obtained to confirm a reduction in hERG protein at the cell membrane with fluoxetine or norfluoxetine (*cf* Cordes *et al*., 2005), but a loss of functional hERG channels by incubation in fluoxetine was demonstrated electrophysiologically. It seems safe to conclude, therefore, that fluoxetine and norfluoxetine do indeed impair hERG trafficking, particularly when the present data are considered alongside independent findings for fluoxetine made recently using an antibody-based chemiluminescent assay (Wible *et al*., 2005). The precise mechanism by which these drugs inhibit trafficking remains to be established, but if it involves a direct action of the drugs on the hERG channel protein, the binding site must differ from that which mediates acute I_{hERG} block. This is because the acute effects of fluoxetine were highly sensitive to mutation of the S6 amino-acid residue Phe-656 (which forms a key part of the binding site for the majority of drugs that block hERG; Sanguinetti and Mitcheson, 2005; Sanguinetti and Tristani-Firouzi, 2006), but disruption of trafficking was unaffected by this mutation. Moreover, a high concentration of the potent hERG blocker E-4031, which binds at Phe-656 (Milnes *et al*., 2003; Kamiya *et al*., 2006), was unable to prevent fluoxetine- or norfluoxetine-mediated disruption of trafficking. It is intriguing that although some compounds, including E-4031, can pharmacologically rescue trafficking of some LQT2 mutants (Delisle *et al*., 2004), for other compounds such as fluoxetine and norfluoxetine, disruption of channel trafficking appears likely to contribute to the genesis of QT_CI prolongation.

The wider relevance of this study relates to the accurate identification during the drug design and discovery process of NCEs that impair hERG channel function. Compounds producing acute hERG channel blockade are likely to be identified by standard hERG assays, but trafficking effects are

likely to be missed. In a recent screen for drug-induced suppression of hERG, a surprisingly large proportion ($\sim$40%) of hERG blockers demonstrated trafficking effects in addition to pharmacological block (Wible *et al*., 2005). Thus, standard hERG assays alone could lead to an underestimation of the net effect on hERG of a drug that combines pharmacological blockade with disruption of trafficking and could overlook entirely an NCE that impaired hERG function due solely to an effect on trafficking. The extent to which this is a real as opposed to theoretical problem remains to be established, although at least six non-blocking hERG trafficking inhibitors are already known to exist (Wible *et al*., 2005). This adds further to the complexity of screening NCEs for QT_CI-prolonging potential and may underscore the importance both of determining chronic effects of promising candidates on the QT_CI using *in vivo* models and the value of *in vitro* assays (e.g Wible *et al*., 2005) able to detect both hERG channel inhibition and drug effects on trafficking.

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