**hERG potassium channel inhibition by ivabradine requires channel gating**

**Dario MelgariMSc1, Kieran E. Brack PhD2, Yihong Zhang PhD1, Aziza El Harchi PhD1, John S. Mitcheson PhD4, Christopher E. Dempsey PhD5, G André Ng MBChB, PhD, FRCP2,3, Jules C. Hancox PhD, FSB, FBPhS1\***

1 School of Physiology & Pharmacology, Medical Sciences Building, University Walk, Bristol, BS8 1TD. UK

2 Department of Cardiovascular Sciences, Cardiology group, University of Leicester, Glenfield Hospital, Leicester, LE3 9QP, UK

3 NIHR.Leicester.Cardiovascular.Biomedical.Research.Unit, Leicester, LE3 9QP, UK

4 Department.of.Cell.Physiology.and.Pharmacology, Maurice Shock Medical Sciences Building, University Road, PO Box 138, Leicester, LE1 9HN, UK

5 School.of.Biochemistry, Medical Sciences Building, University Walk, Bristol, BS8 1TD, UK

\* Correspondence to:

Professor Jules C Hancox, School of Physiology & Pharmacology, Medical Sciences Building, University Walk, Bristol, BS8 1TD, UK. Tel +44-(0)117-3312292; Fax +44-(0)117-3312288; email: [jules.hancox@bristol.ac.uk](mailto:jules.hancox@bristol.ac.uk%7d)

We read with great interest the recent paper in the journal by Lees-Miller and colleagues, who have demonstrated that the clinically used specific bradycardic agent ivabradine inhibits hERG potassium channels at concentrations overlapping those that inhibit HCN4, the major HCN isoform in the mammalian sinoatrial node [1]. They further demonstrated inhibition by the drug of the native rapid delayed rectifier K+ current, IKr, from murine fetal ventricular myocytes and that it delayed ventricular repolarization in those cells [1]. These findings complement those in a recently published report from our laboratories, also reporting hERG K+ channel inhibition and delayed ventricular repolarization with ivabradine, the latter observed in adult guinea-pig perfused intact hearts [2]. We also observed changes to refractory period and steepening of the left ventricular basal action potential restitution curve with the drug [2], effects that are associated with increased risk of ventricular fibrillation. Ivabradine has been thought to have a good cardiac safety profile [3], but within the last year the drug has been added to the “CredibleMeds” database of drugs associated with QT prolongation and *Torsades de Pointes* (TdP) as carrying a conditional risk of TdP [4], and case reports of TdP in patients receiving ivabradine together with other medications have begun to appear [5,6]. The characterization of hERG interactions of the drug is therefore timely.

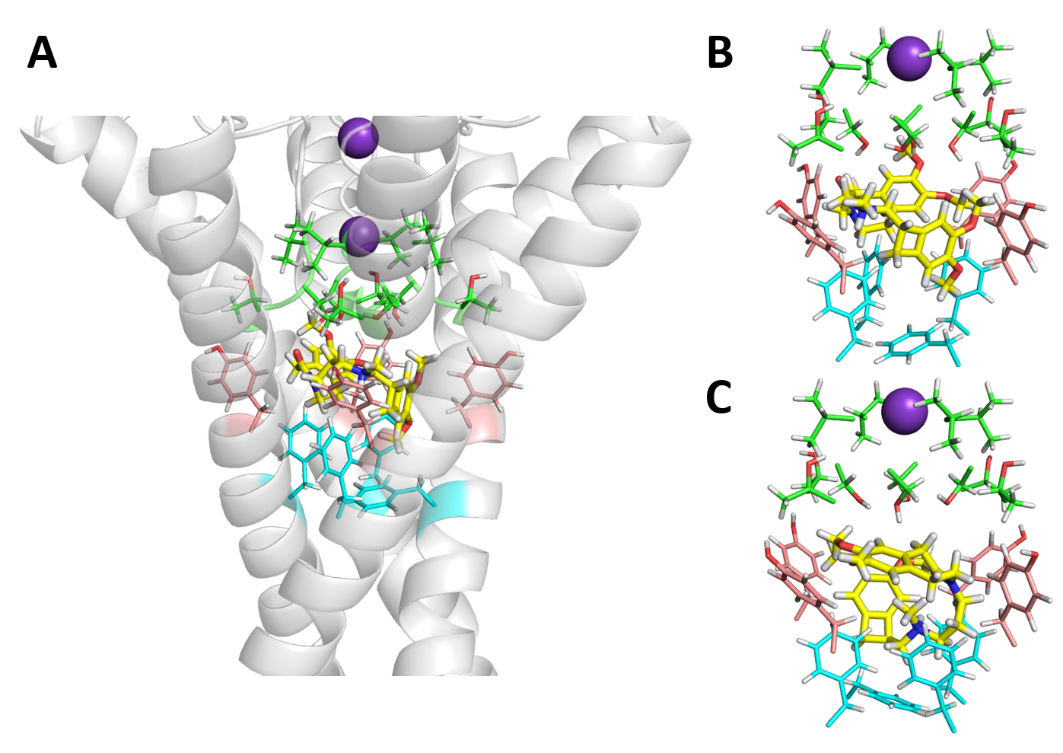
In the Discussion of their paper, Lees-Miller and colleagues highlight some similarities and differences between their findings [1] and our own recent report [2]: both studies provide evidence for ivabradine binding within the inner cavity of the channel and for interactions with the aromatic residues that are widely accepted as forming key components of the canonical binding site. From alanine mutagenesis and *in silico* docking we reported drug interactions with both Y652 and F656 residues [2]. Lees-Miller and colleagues reported strong interactions with Y652, but in using different mutations at F656 saw little (F656I) or only modest (F656C) attenuation of drug block [1]. In addition, through the use of molecular dynamics simulations in which the channel was placed in a lipid environment [1], Lees-Miller and colleagues demonstrated potential for drug interactions with a second novel site in a lipid-exposed pocket (formed largely by F551, I663, M651 and L622 in the open state and by the side-chains of V644, L552 and F551 in the closed state). They additionally simulated ivabradine solubilization into lipid bilayer, showing a strong propensity for the drug to partition into the lipid, and proposed a lipophilic route for drug access to the channel [1].

These results are intriguing, and the notion that the drug may accumulate in lipid is attractive, because at clinically relevant plasma levels of the drug [7] comparatively little inhibition of either HCN4 or hERG channels might be anticipated and yet the drug is clearly clinically effective at slowing heart rate. Interesting questions arise, however, as to the likely contribution of the second novel potential interaction site and regarding the gating dependence of the drug’s inhibitory effect on hERG. Lees-Miller *et al* reported tonic block of IhERG using a protocol in which a gap was introduced between application of a large concentration of drug (20 µM) and voltage-protocol application, with maximal block then occurring on the first pulse in the presence of drug [1]. Two possible interpretations of this result are (i) that once the drug has accumulated at its binding site(s) it interacts with and inhibits channels in the closed/resting state; (ii) that once the drug has accumulated, it binds rapidly when the channel opens on membrane potential depolarization. We favor the second interpretation. In our study, we applied an ‘envelope of tails’ protocol to investigate gated versus resting/closed state channel block [2], using a drug concentration (3 µM) close to our observed IC50. For very brief depolarizing commands little inhibition was observed, with block developing rapidly with progressively longer depolarizations (with a measured time-constant at 37oC of 111 ms for inhibition of tail currents following depolarizations to +20 mV). Thus, under our experimental conditions, at least, any component of closed channel block of hERG by ivabradine was very small or absent. Additional evidence that channel gating is required for ivabradine to inhibit the channel comes from the very substantial decrease in inhibitory potency observed by Lees-Miller *et al* for the S620T inactivation-deficient mutant [1]. As the S620 residue does not form a direct interaction with the drug, this mutation most likely exerts its effects through inactivation-gating dependent conformational rearrangements, in which those residues that do interact with the drug become optimally positioned as a result of inactivation gating [8]. Two other observations also support gating dependence of block. We observed marked positive-voltage-dependence of IhERG inhibition at 37oC by ivabradine, with the range of voltage-dependence overlapping the ascending region of the voltage-dependent activation relation for IhERG; this is consistent with activation-dependence of inhibition [2]. Some voltage-dependence of inhibition is also evident in Figure 2C of the study of Lees-Miller and colleagues as the current-voltage relations for peak tail current in control and ivabradine only diverge substantially at 0mV and more positive voltages [1]. Preferential closed channel block would likely exhibit either neutral or inverse-voltage dependence (e.g. [9]). Finally, we observed a modest decrease in blocking potency of the drug when the direction of IhERG (and thus K+ flux) was reversed with high external [K+] [2]. This is consistent with the potential for some interaction between K+ ions flowing inward through the channel and the drug; for that to occur the drug must be positioned somewhere in the ion permeation pathway. In this regard it is of interest that the hERG IC50 found by Lees-Miller and colleagues (of 6.8 µM) measuring inward IhERG tails was higher than that for native IKr (2.4 µM) from outward tail current measurements in their study [1].

The Y652 residue faces the hERG channel inner cavity and it is significant that the Y652A mutant had a very large effect on IhERG block in the study by Lees-Miller and colleagues. Despite the evidence in their study for a second potential interaction site, they conclude that the intra-cavity binding site is likely to be favoured by the drug [1]. We fully agree with this conclusion. Additionally, if the drug does access the cavity via a lipophilic route, either that access and/or consequent binding and inhibition require channel gating to occur. The results from our ‘envelope of tails’ experiment can only be accounted for by drug association with its target residues proceeding when the channel gates. We have performed docking simulations of ivabradine to a KcsA-based homology model of the closed hERG channel [10, 11] (Figure 1). Whilst the channel’s inner cavity can certainly accommodate ivabradine in the closed state, occupation of the closed-state cavity by the drug necessitates close approaches between drug and key aromatic residues, in particular, Y652 (Figure 1). A dominance of closed channel block resulting from binding of ivabradine within the closed state cavity would therefore likely be reflected by substantial inhibition at very early time-points during the envelope of tails protocol, which was not observed in our study [2]. Thus, it seems most likely that, whether via a lipophilic route or from the cell interior after crossing the membrane, ivabradine enters and inhibits the channel at the inner cavity site when the channel activates, with inactivation helping stabilize/optimize drug binding.

While the study by Lees-Miller and colleagues [1] and our own [2] are not in agreement in all aspects, both reports show clearly that ivabradine blocks hERG channels at concentrations overlapping those that inhibit HCN4/If. Collectively, the findings of both studies highlight the need for rigorous post marketing surveillance of this drug. Additionally, potential drug access to the hERG channel inner cavity through a lipophilic route has far-reaching consequences beyond ivabradine alone and is an area that warrants detailed study. We congratulate Lees-Miller and colleagues on their valuable and thought-provoking paper.

**Acknowledgement**: The authors thank the British Heart Foundation for research funding.



**Figure Legend**

**Figure 1. Docking simulation of ivabradine binding to a closed channel hERG model.**

**(A)** Representative low-energy score pose of ivabradine docked to a hERG closed-state homology model [11] built on a closed state KcsA structure (pdb: 1BL8). Pore and S6 helices are shown as grey ribbons, whilst relevant pore cavity residues are shown as sticks: T623, S624 and V625 (green), Y652 (pink), F656 (blue). Ivabradine is represented as yellow sticks, whilst the purple spheres represent K+ ions in position S1 and S3 of the selectivity filter. **(B, C)** Two GOLD low-energy score poses for ivabradine docked to the KcsA-based homology model. Colour representations are as described for **A**. The restricted dimensions of the closed state cavity forces ivabradine to lie close to the side chains that line the cavity. In particular, ivabradine tends to interact with Y652 side chains whether the molecule is docked to the higher **(B)** or lower **(C)** region of the pore cavity. For references to colour in the figure, see the online version of this article.

**References**

1. Lees-Miller JP, Guo J, Wang Y, Perissinotti LL, Noskov SY, Duff HJ. Ivabradine prolongs phase 3 of cardiac repolarization and blocks the hERG1 (KCNH2) current over a concentration-range overlapping with that required to block HCN4. J Mol Cell Cardiol 2015; 85:71-78.

2. Melgari D, Brack KE, Zhang C, Zhang Y, El Harchi A, Mitcheson JS, et al. hERG potassium channel blockade by the HCN channel inhibitor bradycardic agent ivabradine. J Am Heart Assoc 2015; 4: pii: e001813. doi: 10.1161/JAHA.115.001813.

3. Savelieva I, Camm AJ. Novel If current inhibitor ivabradine: safety considerations. Adv Cardiol 2006; 43: 79-96.

4. Anon. https://www.crediblemeds.org/blog/nelfinavir-and-ivabradine-added-list-drugs-avoid/ Accessed June 05 2015

5. Cocco G, Jerie P. Torsades de Pointes Induced by the Concomitant Use of Ivabradine and Azithromycin: An Unexpected Dangerous Interaction. Cardiovasc Toxicol 2015; 51: 104-106.

6. Mittal SR. Slow junctional rhythm, QTc prolongation and transient torsades de-pointes following combined use of Ivabradine, Diltiazem and Ranolazine. J Assoc Physicians India 2014; 62: 426-427.

7. Jiang J, Tian L, Huang Y, Li Y, Xu L. Pharmacokinetic and safety profile of ivabradine in healthy Chinese men: a phase I, randomized, open-label, increasing single- and multiple-dose study. Clin Ther 2013; 35: 1933-1945.

8. Chen J, Seebohm G, Sanguinetti MC. Position of aromatic residues in the S6 domain, not inactivation, dictates cisapride sensitivity of HERG and eag potassium channels. Proc Natl Acad Sci USA 2002; 99: 12329-12333.

9. Milnes JT, Dempsey CE, Ridley JM, Crociani O, Arcangeli A, Hancox JC, et al. Preferential closed channel blockade of HERG potassium currents by chemically synthesised BeKm-1 scorpion toxin. FEBS Lett 2003 ; 547: 20-26.

10. Duncan RS, Ridley JM, Dempsey CE, Leishman DJ, Leaney JL, Hancox JC, et al. Erythromycin block of the HERG K+ channel: accessibility to F656 and Y652. Biochem Biophys Res Comm 2006; 341: 500-506.

11. Witchel HJ, Dempsey CE, Sessions RB, Perry MD, Milnes JT, Hancox JC, et al. The low-potency, voltage-dependent HERG channel blocker propafenone - molecular determinants and drug trapping. Mol Pharmacol 2004; 66: 1201-1212.

12. Doyle DA, Morais Cabral J, Pfeutzner RA, Kuo A, Gulbis JM, Cohen SL, et al. The structure of the potassium channel: molecular basis of K+ conduction and selectivity. Science 1998; 280: 69-77.