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COMMENTARY

The 'overly-sensitive' heart: sodium channel block and QRS interval prolongation

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Cardiac safety remains of paramount importance in the development of successful clinical drug candidates. Great progress has been made recently in understanding liabilities associated with delayed ventricular repolarization (manifest as QT prolongation) and in predicting (thus avoiding) drugs that delay repolarization based on application of strategic preclinical assays. Following the advances made in clinical electrophysiological monitoring and conduct of thorough QT studies, focus is now shifting towards monitoring of additional drug-induced effects, particularly on ventricular conduction (measured as changes in the QRS interval on the ECG) as part of evolving clinical thorough ECG studies. In this issue of the *British Journal of Pharmacology*, a study by Harmer *et al.* proposes provisional safety margins for QRS prolongation in man based on retrospective clinical data and a single *in vitro* approach to assess potency of block of cardiac sodium current (hNav1.5), the ionic current responsible for ventricular conduction (observed as QRS prolongation). The present commentary places their study in context with evolving preclinical cardiac electrophysiological safety assessments, along with discussions focused on ensuring the proper 'translation' of preclinical findings with potential clinical concerns. Given the extant limitations and uncertainties of presently available data, as well as our limited understanding of the pro-arrhythmic potential associated with these changes, due caution should be applied when considering the proposed *in vitro*-based margins for drug-induced QRS prolongation measured clinically. Additional validation with multiple preclinical models and more rigorous clinical safety studies will be necessary to substantiate these recommended margins.

LINKED ARTICLE

This article is a commentary on Harmer *et al.*, pp. 260–273 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2011.01415.x

Abbreviations

Cmax, maximal drug concentration; ECG, electrocardiogram; FDA, Food and Drug Administration; $\dot{g}_{Na}$, maximal sodium current conductance; hERG, human ether a-go-go related gene; hNav1.5, human cardiac sodium channel; ICH, International Conference on Harmonisation; IC_{50}, concentration eliciting 50% inhibition; I_{Kr}, rapidly activating inward rectifier current; Na, sodium; QT, QT interval on the ECG; QTc, QT interval corrected for heart rate; QRS, QRS interval on the ECG

The current drug development paradigm used by pharmaceutical companies necessitates a critical understanding regarding the translation of preclinical findings to clinical study findings in order to discard potentially unsafe drug candidates while ensuring the safety of clinical trial volunteers and patients. However, it is also important to minimize the high rate (~86%) of late stage attrition of compounds due primarily to clinical safety concerns, lack of efficacy, poor pharmacokinetic profiles and toxicological issues (Frank and Hargreaves, 2003; Shah, 2006) in order to develop needed novel therapeutics. This is particularly important for untoward (and usually 'off-target') cardiovascular effects, including those related to cardiac function. While the past few years have seen enormous efforts directed at understanding (and avoiding) effects related to delayed cardiac repolarization (often the consequence of blockade of the fast component of the delayed rectifier current, I_{Kr}, resulting in QT/QTc interval prolongation), emphasis may now be shifting to better understand the liabilities associated with drug effects on cardiac conduction.

On the surface ECG, the QRS interval reflects ventricular depolarization and propagation of the excitatory cardiac impulse throughout the ventricles. On a cellular level, depolarizing inward current passing through the voltage-gated cardiac sodium (Na) channel ($hNa_V1.5$, the gene product of SCN5A) is responsible for the rapid upstroke (phase 0) of the ventricular action potential that initiates conduction of the excitatory wavefront throughout the ventricular wall. The fast kinetics and high current density of cardiac Na current ensures rapid upstroke velocities of the action potential (typically 250 $V \cdot s^{-1}$ for ventricular myocardium), rapid conduction (~2–4 $m \cdot s^{-1}$) and a high safety margin for propagation. It should be recognized, however, that cardiac conduction is a complex, multifactorial process that is affected by the magnitude of depolarizing current, the extent of cell-to-cell communication through gap junctions, as well as cell and tissue architecture and morphology. A change in ventricular conduction is manifest as prolongation of the QRS interval on the ECG, and drug-induced effects on ventricular conduction are nearly always associated with block of cardiac Na current.

The translation of *in vivo* preclinical drug safety findings to clinical safety results has always been a challenging endeavour and particularly important with regards to issues surrounding cardiac safety. This has been amply demonstrated with delayed repolarization, a surrogate marker of pro-arrhythmia manifest clinically as prolongation of the QT interval. To guard against drug-induced delayed repolarization, minimally, two preclinical assays are routinely used to detect the potential for a pro-arrhythmic liability, namely an *in vitro* hERG electrophysiology assay (that measures the important cardiac I_{Kr} repolarizing current) as well as an *in vivo* ECG-based assay [that measures changes in the QT interval (International Conference on Harmonization (ICH) S7B Guideline; US FDA, 2005a)]. These preclinical findings can then be compared to clinical findings derived from the conduct of a clinical thorough QT study. An initial retrospective study benchmarking hERG assay performance was recently published (Gintant, 2011), and further evaluations considering both the *in vitro* and *in vivo* assays to evaluate concordance between signals are ongoing (Trepakova *et al*., 2009). Despite improved precision and fidelity of recording methods used to define drug-induced effects in preclinical assays, it is still difficult to translate these findings to clinical experience, linking pharmacokinetic and pharmacodynamic responses.

A recent study by Lu *et al*. (2010) demonstrated that approximately one-third of 355 compounds tested had an effect on cardiac conduction defined as a ≥15% reduction in the maximum rate of rise of the upstroke velocity, V_{max}, recorded in rabbit isolated Purkinje fibres or an increase in intraventricular conduction time in rabbit Langendorff hearts, or ≥10% widening of the QRS duration in left-ventricular wedge preparations or isolated hearts (Lu *et al*., 2010). However, current ICH guidelines do not address drug-induced prolongation of the QRS duration or slowed conduction as an independent predictor for the development of ventricular arrhythmias. Indeed, the recent voluntary withdrawal of propoxyphene (an opioid analgesic used to treat mild to moderate pain) from the US market resulted from a review of data that showed the drug had the potential to induce cardiac conduction disturbances and impart ECG changes (at therapeutic doses) including PR interval prolongation, a widened QRS complex and a prolonged QT interval. Based upon these changes propoxyphene may have the potential to increase the risk for serious abnormal heart rhythms, especially in patients with ischaemic heart disease (US FDA Drug Safety Communication, 2010).

The study by Harmer *et al*. (2011 – this issue) explored only the relationship between drug-induced block of cardiac Na current *in vitro* with clinical literature reports of QRS prolongation. This group evaluated the blocking potency of 98 compounds for which they were able to determine IC_{50} values against the human cardiac Na current ($hNa_V1.5$) using an automated patch clamp system ($hNa_V1.5$ IonWorks™ assay). They then calculated safety margins for these drugs by comparing the IC_{50} values for Na current block with calculated free plasma concentrations from clinical studies that measured QRS prolongation. The data provided evidence of a provisional safety margin of 30- to 100-fold between $hNa_V1.5$ IC_{50} and the free C_{max} values (referred to as fC_{max}) conferring an acceptable degree of safety from QRS prolongation. However, QRS prolongation occurred, on average, at free plasma levels 15-fold below the $hNa_V1.5$ IC_{50} values. Interestingly, this provisional margin is similar to that proposed previously by Redfern *et al*. (2003) defining a provisional early hERG safety margin.

Perhaps the most surprising finding of this study is that QRS widening occurs at concentrations well *below* the IC_{50} values for block of $hNa_V1.5$ current measured with heterologous expression systems in automated patch clamp platforms. Given the high current density of native $hNa_V1.5$ and the lack of opposing overlapping currents during the action potential upstroke, one might expect a high 'depolarization margin', with modest block of cardiac Na current having minimal effect on intraventricular conduction. However, the work by Harmer *et al*. (2011) would argue the contrary: QRS prolongation should be anticipated with some drugs at concentrations eliciting only ~10–20% block of $hNa_V1.5$. These results are not unlike the low 'repolarization margin' often used for hERG repolarizing current, where a small decrease in a low density (but influential) repolarizing current can elicit substantial and physiologically significant delays in ventricular repolarization.

Some prior studies also suggest that small reductions in cardiac Na current will affect QRS widening. A recent study by Heath *et al*. (2011) concluded that QRS interval prolongation (~10–20%) observed in either preclinical or clinical studies with flecainide and mexiletine occurred at free plasma concentrations 6- to 30-fold below IC_{50} values for block of $hNa_V1.5$ (Heath *et al*., 2011). In addition, an earlier abstract by Cordes *et al*. (2009) comparing the magnitude of *in vitro* human cardiac $hNa_V1.5$ current inhibition required to modify QRS duration *in vivo* concluded that free plasma concentrations equivalent to a fraction (actually ~3- to 11-fold below) the IC_{50} value of Na current block are sufficient to produce QRS widening (Cordes *et al*., 2009). Computer simulation studies of cardiac propagation using multicellular models described a non-linear relationship between conduction velocity and reduced maximal Na current conductance ($\dot{g}_{Na}$); for example, a 20% decrease in $\dot{g}_{Na}$ minimally reduced conduction velocity from 55 to 50 $cm \cdot s^{-1}$ while a 50% decrease in $\dot{g}_{Na}$ reduced conduction velocity from 55 to only

42 cm·s^{-1} (Shaw and Rudy, 1997). While the relationship between changes in conduction velocity and QRS widening was not determined, the findings predict that small reductions in Na current will minimally affect intraventricular conduction. This study also noted that the safety factor for conduction (i.e. the ratio of charge generated by cell excitation vs. the minimal amount of charge required to cause excitation of adjacent elements) declined prominently only after $\dot{g}_{Na}$ was reduced by greater than 80%, suggesting that *extreme* QRS prolongation is necessary for intraventricular conduction failure in normal hearts. Indeed in cases of overdose with tricyclic antidepressants (many shown to block cardiac Na current at high exposures), ventricular conduction is sustained despite QRS widening by nearly twofold in some patients (Hultén *et al.*, 1992; Choi and Lee, 2008).

As with any scientific study, a number of limitations of the work by Harmer *et al.* (2011) are noted (Table 1). One area of concern relates to the validity of the IC_{50} values determined for drug-induced block of $hNa_V1.5$ in relation to the *in vivo* reduction of cardiac Na current. Currents were recorded from $hNa_V1.5$-expressing CHO cells using the Population Patch Clamp™ mode of the IonWorks™ automated planar patch clamp electrophysiology platform. This approach records averaged (ensemble) ionic currents from a population of up to 64 cells expressing the recombinant voltage-gated Na ion channel (Harmer *et al.*, 2008), optimizing the moderate throughput of this instrument. As this instrument does not provide for voltage control between control and drug-test voltage clamp protocols, it is possible that unanticipated drug binding may have occurred between the voltage clamp pulses that, in turn, affect the potency determinations. Utilizing this assay, the group also did not consider the effects of voltage- or use-dependent block, which may dramatically alter the determination of potency of block for some compounds (Wang *et al.*, 1996). The variability of derived IC_{50} values (both within-lab and between laboratories) must also be considered. An earlier study comparing potency of hERG current block across two laboratories reported interlaboratory variability for IC_{50} values generally about threefold or less (with an exception of one compound where a 40-fold difference was noted) using the same source compounds and study protocols (Hanson *et al.*, 2006). In the present situation, Harmer *et al.* (2011) report IC_{50} values for Na current block by flecainide and mexiletine of 5.8 and 25.7 μM respectively, while Heath *et al.* (2011), reported corresponding values of 10.7 and 67.2 μM using the same patch platform (but different voltage clamp protocols). As a consequence, the calculated safety margins for flecainide from Harmer *et al.* (2011) and Heath *et al.* (2011) are 14.7 versus 27.1, respectively, while for mexiletine values of 4.7 versus 12.4 are obtained from the two groups. Indeed, from the two free C_{max} values reported for mexiletine by Harmer *et al.* (2011), the calculated safety margin ranges from 2.6 to 60 depending on the IC_{50} values used. A comparable wide safety margin range (8–53) for flecainide is derived from the broad range of clinical free plasma C_{max} values from multiple studies summarized by Heath *et al.* (0.2–1.35 μM) along with their mean IC_{50} value. Such differences highlight potential variability across laboratories and clinical studies, the utility of interval estimates (such as the confidence intervals) when interpreting narrow therapeutic margins, and emphasize the importance of cautious interpretation of such provisional guidelines.

It is also well known that blockade of cardiac Na current is modulated by rate, holding potential, and test pulse poten-

Table 1

Multiple limitations affecting the translation of preclinical 'QRS signals' to clinical QRS widening

Challenges in translating preclinical QRS 'signals' to clinical QRS widening
Preclinical:
***In vitro*: functional sodium current studies**
Preparation: hNaV1.5 (contribution of subunits, metabolic modulation)
Experimental conditions affecting potency determinations:
Voltage clamp protocol
• holding potential influences tonic block
• stimulation rate influences use-dependent block
• duration and voltage of test pulse (not physiological)
Patch Clamp approach (tight, loose, population)
Bath exposures – loss of compound due to absorption, etc.
Temperature (room vs. physiological temperature)
Relationship between current block and conduction slowing
Reproducibility/reliability of results across labs, platforms
Testing of cardioactive metabolites (*if present*)
Effect of plasma protein binding on effective free drug concentration
***In vivo*: ECG studies**
Myocardial drug accumulation
Heart rate (influence on use-dependent block)
Exposure (ideally, for pharmacokinetic/pharmacodynamic modelling)
Study power (to detect QRS difference)
Screening efforts probably reduce number of drugs affecting ventricular conduction
Clinical:
Availability of blinded, placebo-controlled, baseline-adjusted studies
Adequate QRS duration assessment
Study power (to detect QRS difference)
Matching exposure determinations (for pharmacokinetic/pharmacodynamic modelling)
Biased reporting
Drug that affect QRS may not be acknowledged, reported
Drugs not affecting QRS (or ECG) not likely to be published
Evolving screening efforts may limit drugs likely to prolong QRS
Pharmacological versus physiological 'significance' of QRS widening

The table considers only *in vitro* voltage clamp approaches and does not include consideration of either *in vitro* action potential (upstroke or conduction) studies or isolated heart (QRS, conduction) studies.

tial (Hondeghem and Katzung, 1977), and that potency of steady-state (or tonic) block may differ substantially from use-dependent block measured with rapid repetitive pulses that promote drug-channel association. In contrast, some recent data suggest that differences in block potency (at least for some drugs) may not be that different *in vitro*. A study by Penniman *et al*. (2010) compared tonic and use-dependent block of cardiac Na current for lidocaine, mexiletine and flecainide using the PatchXpress automated patch platform (that provides for continuous control of voltage throughout the protocol) and conventional voltage clamp methods. While demonstrating differences in absolute potency, these differences were minor, generally threefold or less when determined at similar holding potentials using identical pulse protocols. Thus, while potency values are expected to vary with different voltage clamp protocols (by >20-fold), these differences may not be large enough to invalidate the broad provisional margin (30- to 100-fold) suggested by Harmer *et al*. (2011). However, the extent to which potency *in vitro* may differ from *in vivo* potency as a result of differences in electrical activity (holding potential, test pulse potential and duration, diastolic duration, etc.) is uncertain. In addition, the extent to which potency of block differs between room temperature (where most automated systems perform) and physiological temperatures is uncertain. These issues arise as the native cardiac Na current is difficult (but not impossible, see Murray *et al*., 1990; Berecki *et al*., 2010) to measure under physiological temperatures and conditions due to rapid kinetics and high current density. It should be noted that differences in potency of block with temperature have been observed with the active metabolite of the local anaesthetic-type anti-arrhythmic agent encainide (Johns *et al*., 1989).

As mentioned by Harmer *et al*. (2011), their study was also limited by availability of precise clinical data that provided the QRS interval values along with drug exposure information. While making it difficult to estimate the therapeutic window between $hNa_V1.5$ block potency and clinical exposures causing QRS prolongation, this represents the best available data to date. Furthermore, data were obtained from multiple clinical sources, including case studies (that included drug overdose and studies from patients with cardiac disease) as well as some studies conducted in healthy, normal volunteers. As it is likely that the QRS widening observed in patients with ischaemic cardiac disease may be greater than that observed in healthy individuals, this study group heterogeneity confounds the analysis. Finally, preclinical and clinical studies both demonstrate greater drug-induced QRS widening at faster versus slower heart rates (Nattel, 1985; Sadanaga *et al*., 1993), consistent with use-dependent block of Na current observed from *in vitro* studies. The greater slowing of ventricular conduction at faster rates will also shift the therapeutic window for QRS prolongation towards lower values. Unlike clinical trials from the past, the continued conduct of thorough QT studies [as per the ICH E14 guideline (US FDA, 2005b)], will now also rigorously monitor both drug-induced PR and QRS interval changes and provide for the emergence of more comprehensive clinical data that firmly define therapeutic margins for QRS prolongation.

Slowed intraventricular conduction, manifest as QRS widening, is generally considered a potential cardiac safety liability. This notion is supported by results from earlier pharmacological clinical trials that suggested that cardiac Na current block may be pro-arrhythmic. Indeed, strategies targeted to reduce the incidence of premature ventricular contractions with class Ic anti-arrhythmic agents such as flecainide and encainide (both known to reduce cardiac Na current and thereby reduce cardiac excitability) formed the basis of the Cardiac Arrhythmia Suppression Trial in the setting of post-myocardial infarction and ventricular ectopy in the late 1980s (Pugsley, 2002). Unfortunately, rather than showing anti-arrhythmic efficacy, the trial demonstrated an association of encainide, flecainide and moricizine with increased mortality and sudden cardiac death, resulting in the premature discontinuation of one arm of the trial (Echt *et al*., 1991). Blockade of cardiac Na current in the setting of impaired and heterogeneous conduction has been implicated in the unintended pro-arrhythmia effect with flecainide (Ranger and Nattel, 1995). Thus, reduction of cardiac Na current is considered as a liability for novel drug candidates, especially in the setting of such predisposing factors as structural heart disease (ischaemia, myocardial infarction, congestive heart failure, left ventricular hypertrophy) genetic factors and high heart rates. A recent genome-wide association study identified 22 loci associated with QRS duration involving genes in pathways with established roles in ventricular conduction (Na channels, transcription factors, calcium-handling proteins) as well as previously unidentified biological processes that include kinase inhibitors (Sotoodehnia *et al*., 2010). Thus, it remains to be seen (as was demonstrated with cardiac potassium currents) whether specific populations may be more susceptible to Na channel blockade.

A study by Desai *et al*. (2006) provided evidence for QRS duration in a general medical population as a significant and independent predictor of cardiovascular mortality (Desai *et al*., 2006). In this study of 44 280 patients (mean age 56 years, 90% men lacking structural heart disease), an 18% increase in cardiovascular risk was noted for every 10 ms increase in QRS duration; those with a QRS duration greater than 130 ms experienced nearly twice the risk of cardiovascular death compared with those with a QRS duration of ≤110 ms. Similarly, Adesanya *et al*. (2008) showed that in patients with reduced cardiac conduction (right bundle branch block), every 10 ms increase in QRS duration was associated with a 26.6% increase in mortality. QRS duration was also found to be an independent predictor of mortality in patients with congestive heart failure (Luliano *et al*., 2002). It is recognized that the mechanisms contributing to non-pharmacological QRS widening (i.e. those associated with coronary artery disease, hyperlipidaemia, chronic obstructive pulmonary disease and cardiomyopathy) are probably different from those elicited by drugs acutely reducing cardiac Na current. Thus, the developed clinical consequences may differ significantly.

Conclusion

The study by Harmer *et al*. (2011) represents a sound first attempt to translate findings from a preclinical *in vitro* functional assay (Na current) to prior clinical study results (QRS widening) for a potential pro-arrhythmic liability. Their pro-

posed provisional margin highlights two challenges that must be confronted: (i) accurate translation of developed safety margins; and (ii) the physiological significance of QRS widening in normal (and also diseased) hearts. The challenge for the pharmaceutical industry is to now clarify whether or not a safety margin for drug-induced QRS prolongation can, or should, be proposed. Given the uncertainty in presently available preclinical and clinical datasets as well as the different electrophysiological substrates that are likely to be present in various patient populations, the provisional safety margin of 30–100 would appear to provide a *speculative* target for potency values derived using automated patch clamp systems. However, this margin must be balanced against unwarranted early attrition of potentially highly efficacious preclinical candidates for terminal disease states.

Although a brave and admirable review of data has been provided by the authors, readers should not readily adapt these criteria as 'dogma'; rather, the data obtained and reviewed by Harmer *et al*. (2011) should be the starting point for validation studies that evaluate the relationship between drug-induced Na channel block, slowed conduction (i.e. QRS interval prolongation) and pro-arrhythmic liability associated with these effects. Thus, Na channel blockade and mechanisms responsible for pro-arrhythmic liability require the same degree of investigation as was needed for drug-induced QT prolongation. In a manner similar to that proposed for integrated preclinical QT studies, earlier *in vivo* investigations of QRS widening could complement and further our understanding of *in vitro* functional current studies to de-risk compounds early in drug discovery.

With hERG 'signals' being filtered early from drug discovery pipelines, and thorough QT studies now routinely monitoring PR and QRS intervals, it is likely that drug effects on cardiac conduction (and contractility) will assume a more prominent role in preclinical evaluation of cardiac liabilities and therefore lead to a better understanding in this area. Greater discussion between industry, academia and regulatory authorities, preclinical model validation and development of a clinical drug database are needed to address these important questions.

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Conflict of interest

All authors are involved in preclinical pharmaceutical safety initiatives that impact the entire industry. This drug safety review involves no conflicts of interest.

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