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RESEARCH PAPER

On the relationship between block of the cardiac Na^+ channel and drug-induced prolongation of the QRS complex

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BACKGROUND AND PURPOSE
Inhibition of the human cardiac Na^+ channel ($hNa_v1.5$) can prolong the QRS complex and has been associated with increased mortality in patients with underlying cardiovascular disease. The safety implications of blocking $hNa_v1.5$ channels suggest the need to test for this activity early in drug discovery in order to design out any potential liability. However, interpretation of $hNa_v1.5$ blocking potency requires knowledge of how $hNa_v1.5$ block translates into prolongation of the QRS complex.

EXPERIMENTAL APPROACH
We tested Class I anti-arrhythmics, other known QRS prolonging drugs and drugs not reported to prolong the QRS complex. Their block of $hNa_v1.5$ channels (as IC_{50} values) was measured in an automated electrophysiology-based assay. These IC_{50} values were compared with published reports of the corresponding unbound (free) plasma concentrations attained during clinical use (fC_{max}) to provide an $IC_{50} : fC_{max}$ ratio.

KEY RESULTS
For 42 Class I anti-arrhythmics and other QRS prolonging drugs, 67% had $IC_{50} : fC_{max}$ ratios <30. For 55 non-QRS prolonging drugs tested, 72% had ratios >100. Finally, we determined the relationship between the IC_{50} value and the free drug concentration associated with prolongation of the QRS complex in humans. For 37 drugs, QRS complex prolongation was observed at free plasma concentrations that were about 15-fold lower than the corresponding IC_{50} at $hNa_v1.5$ channels.

CONCLUSIONS AND IMPLICATIONS
A margin of 30- to 100-fold between $hNa_v1.5$ IC_{50} and fC_{max} appears to confer an acceptable degree of safety from QRS prolongation. QRS prolongation occurs on average at free plasma levels 15-fold below the IC_{50} at $hNa_v1.5$ channels.

LINKED ARTICLE
This article is commented on by Gintant *et al.*, pp. 254–259 of this issue. To view this commentary visit http://dx.doi.org/10.1111/j.1476-5381.2011.01433.x

Abbreviations
fC_{max}, unbound (free) plasma concentration attained during clinical use; PPB %, human plasma protein binding; [QRS]free, the free drug concentration that is associated with prolongation of the QRS complex in humans; TdP, Torsade de Pointes

Introduction

Drug-induced changes to the electrocardiogram (ECG) are a key concern to both the pharmaceutical industry (Pollard *et al.*, 2008) and regulators (ICH S7A and B, ICH E14: Anonymous, 2000; 2005a,b). This concern largely resulted from the promiscuity of the hERG K^+ channel, and its relationship to drug-induced QT interval prolongation and Torsade de Pointes (TdP). However, drug block of cardiac ion channels other than hERG is beginning to receive some consideration

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(Chen *et al*., 2009). As a result, pharmaceutical companies and contract research organizations have employed screening against a panel of cardiac ion channels as one of the earliest steps of preclinical cardiac safety assessment (Wible *et al*., 2008; Chen *et al*., 2009).

The cardiac Na^+ channel ($hNa_v1.5$, encoded by the SCN5a gene; nomenclature follows Alexander *et al*., 2009) is one such safety target. This channel is primarily responsible for the depolarization of atrial and ventricular myocytes. Blockade of the Na^+ current decreases the rate of depolarization, which in turn slows the velocity of excitation conduction. If the slowing of conduction is large enough, this can be measured as prolongation of the QRS complex on the ECG. Indeed, many drugs that block the cardiac Na^+ channel are associated with QRS prolongation: these include Class I anti-arrhythmics, which block the cardiac Na^+ channel as the primary mechanism of action such as flecainide and propafenone, as well as members of several other classes of non-cardiovascular drugs (e.g. tricyclic antidepressants, antipsychotics, anticonvulsants and antimalarials; Madias, 2008). Furthermore, an analysis of the FDA's Adverse Events Reporting System showed that there were 1194 clinical QRS adverse events reported, attributed to more than 500 drugs, between November 1997 and November 2007 (Valentin, 2010).

The safety implications of blockade of the cardiac Na^+ channel and QRS prolongation remain a matter of debate. Genetic evidence suggests that loss of $hNa_v1.5$ channel activity is associated with numerous cardiac disorders and is often linked with potentially fatal arrhythmias (Tan *et al*., 2003). Furthermore, pharmacological inhibition of the cardiac Na^+ channel may also trigger arrhythmias and can be associated with an increased mortality rate (Echt *et al*., 1991). A prolonged QRS duration has also been linked with an increase in mortality – in patients with right bundle branch block, for every 10 ms increase in QRS duration, the risk of death rises by 26.6% (Adesanya *et al*., 2008). Despite this, the link between QRS prolongation and arrhythmias in healthy individuals is not well understood (Seger, 2006). Nonetheless, the possible safety concerns of cardiac Na^+ channel blockade make it prudent to identify and eliminate this liability early in the drug discovery process. To this end, we have previously described a $hNa_v1.5$ channel assay based on the IonWorks™ planar patch technology (Harmer *et al*., 2008).

Interpretation of the potency of a compound to block $hNa_v1.5$ channels requires knowledge of how $hNa_v1.5$ channel block translates into prolongation of the QRS complex. To explore this relationship, we determined the blocking potencies (IC_{50} values) at $hNa_v1.5$ channels for three categories of drugs: (i) Class I anti-arrhythmics; (ii) other drugs associated with QRS complex prolongation; and (iii) drugs not reported to prolong the QRS complex. Safety margins for these drugs were calculated by comparing the values of IC_{50} at $hNa_v1.5$ channels with published reports of the maximum unbound (i.e. free) plasma concentrations attained during clinical use (fC_{max}). We hope that these data will provide evidence for setting provisional safety margins, and aid in the interpretation of potency data for block of $hNa_v1.5$ channels, obtained during safety screening, early in the drug discovery process.

Methods

$hNa_v1.5$ IonWorks™ assay

Blocking potencies at $hNa_v1.5$ channels (as IC_{50}) were determined using the IonWorks™ assay previously described (Harmer *et al*., 2008). This assay is able to determine $hNa_v1.5$ channel blocking potencies that are in good agreement with those determined using conventional patch clamp, as well as well as predicting Na^+ channel effects in more integrated systems such as the cardiac Purkinje fibre action potential. Briefly, currents were recorded from $hNa_v1.5$ channel-expressing CHO cells using the Population Patch mode of the IonWorks™ device. Experiments were conducted at room temperature. Pre- and post-compound $hNa_v1.5$ currents were evoked by a single voltage train consisting of a 15 s period holding at –90 mV, a 160 ms step to –100 mV (to obtain an estimate of leak current), a 100 ms step back to –90 mV, followed by a series of eight pulses to 0 mV for 50 ms from a holding potential of –90 mV applied at 3 Hz. In between the pre- and post-compound voltage pulses there was no clamping of the membrane potential. Currents were leak-subtracted based on the estimate of current evoked during the step to –100 mV at the start of the voltage pulse protocol. The degree of inhibition of the $hNa_v1.5$ current for each well was assessed by dividing the post-scan $hNa_v1.5$ current by the respective pre-scan $hNa_v1.5$ current for the eighth test pulse. Each compound plate was laid out in 12 columns to enable 10 8-point non-cumulative concentration–effect curves to be constructed; the remaining two columns on the plate were taken up with vehicle (final concentration 0.33% dimethyl sulphoxide), to define the assay baseline, and a supra-maximal blocking concentration of flecainide (final concentration 316 μM) to define the 100% inhibition level. A full concentration-response curve to flecainide was also obtained for each plate to compare to historical data as a quality control measure. Data were normalized to vehicle and 100% blocking levels and the resulting data fitted to the Hill equation using a custom-written Origin-based fitting program (Origin 7.5; OriginLab Corporation, Northampton, MA, USA). The IC_{50} values quoted are the geometric mean of at least two independent experiments, with each test concentration being tested in a minimum of six wells.

Drugs

Redfern *et al*. (2003) have previously described in detail an approach to defining safety margins between the IC_{50}, at hERG channels, and the drug free plasma concentrations and risk of generating TdP. We therefore adopted a similar approach with respect to the safety margins between block of $hNa_v1.5$ channels and drug-induced QRS complex prolongation. Specifically, we have compared $hNa_v1.5$ channel blocking potency data for a range of drugs with known propensity to prolong the QRS complex and compared this to their therapeutic plasma concentrations, as the IC_{50} : fC_{max} ratio in order to establish safety margins.

A list of 132 drugs that are known or suspected to inhibit $hNa_v1.5$ channels was initially compiled. These drugs were sourced either from Sigma-Aldrich (Dorset, UK), Apin Chemicals (Oxford, UK), Tocris Cookson (Bristol, UK) or the AstraZeneca compound collection (Macclesfield, UK).

Assays and data collection

These 132 drugs were then tested in the $hNa_v1.5$ IonWorks™ assay over eight half-log_{10} spaced concentrations. Drugs were tested to the limit of their solubility, up to a maximum test concentration of 100 μM (solubility was assessed by visual inspection). Those compounds that inhibited $hNa_v1.5$ channels by <40% did not have an IC_{50} calculated, and were not analysed further. Information on the remaining 98 drugs was collected and analysed, as described below, and tabulated.

Human plasma protein binding data and therapeutic plasma concentrations were primarily obtained from Redfern *et al*. (2003) and the BIOPRINT™ database (Krejsa *et al*., 2003). Schulz and Schmoldt's (2003) study was also used as a source of therapeutic plasma concentration data, although this publication generally lists trough values at steady state. Other literature sources used are referenced in the tables. Where available, the highest value for the effective therapeutic plasma concentration, or the highest plasma concentration achieved in humans during normal clinical use, has been used for each drug. For the purposes of this paper, this value has been referred to as the C_{max} (maximum clinical concentration). The unbound (free) C_{max} value was calculated using the plasma protein binding value, and is referred to as fC_{max}.

The MEDLINE (PubMed) database was searched for each drug using QRS as an additional search term. The drugs were divided into three categories: (i) Class I antiarrhythmics; (ii) other drugs associated with QRS complex prolongation; and (iii) drugs not reported to prolong the QRS complex (based on a search of the MEDLINE database). A drug was deemed to have changed the QRS complex if the authors concluded that the QRS complex change was noteworthy and drug-related; the magnitude of QRS complex change was not taken into account. For each of the 98 drugs, the ratio of IC_{50} at $hNa_v1.5$ channels to fC_{max} was calculated and plotted on a logarithmic scale. For each drug, the literature was also searched for studies where drug-induced QRS complex prolongation was measured alongside plasma drug concentrations. When possible, the plasma concentration associated with the lowest significant or noteworthy change in QRS complex duration is quoted.

The incidence of false positives/negatives was calculated for a given $IC_{50} : fC_{max}$ ratio as follows: Class I antiarrhythmics and other known drugs associated with QRS complex prolongation were classified as positives, and drugs not reported to prolong the QRS complex were classified as negatives. Then, a specific margin was selected (e.g. 30-fold). A positive compound with an $IC_{50} : fC_{max}$ ratio above 30 was classified as a false positive, a negative compound with an $IC_{50} : fC_{max}$ ratio below 10 was classified as a false negative. This approach was taken for a series of $IC_{50} : fC_{max}$ ratios.

Results

An initial set of 132 drugs was tested in the $hNa_v1.5$ channel assay in order to determine IC_{50} values. The drugs listed below were tested up to the limit of their solubility, up to a maximum test concentration of 100 μM. These drugs were either inactive in the $hNa_v1.5$ assay (i.e. below the normal baseline activity for the assay), or did not inhibit the $hNa_v1.5$ channel sufficiently in order to fit a concentration–effect curve and determine an IC_{50} value:

Alfuzosin, amantadine, amiloride, anthralin, atenolol (S), benzocaine, berberine chloride, betahistine, chlorotrianisene, ciprofloxacin, clobetasol, dofetilide, epirubicin, ethotoin, fampridine, fenoterol, fluorometholone, gabapentin, gabexate, gatifloxacin, ipriflavone, levetiracetam, levocetirizine, levofloxacin, mephenytoin, montelukast, norethindrone, orlistat, phenacemide, d-sotalol, sparfloxacin, tiapride Cl, tocainide HCl and zonisamide. As these drugs were deemed to be inactive at $hNa_v1.5$ channels, they were considered outside the scope of this study and were excluded from further analysis.

Data for the remaining 98 drugs tested in this study are listed in Tables 1, 2 and 3. Table 1 lists Class I antiarrhythmics. Table 2 lists drugs that are associated with prolongation of the QRS complex. Table 3 lists drugs that have not been reported to cause prolongation of the QRS complex. Each shows the $hNa_v1.5$ channel IC_{50} value, molecular weight, plasma protein binding, fC_{max} range and the log_{10} ($hNa_v1.5$ channel IC_{50} : highest fC_{max}). Tables 1 and 2 also show, where data are available, free drug concentrations determined in patients or volunteers where a change in the QRS complex duration has also been measured. The log_{10} of the ratio of IC_{50} to the mean free drug concentration associated with a QRS change (IC_{50} : [QRS]) is also shown. The data contained within the tables formed the basis for further analysis described below. A more comprehensive version of these tables can be found in the supplementary data (Tables S1–S3).

Figure 1 shows examples of $IC_{50} : fC_{max}$ ratio calculations for two drugs. For each drug, the graphs show the concentration–effect curve for blockade of $hNa_v1.5$ channels, as well as the highest fC_{max} value quoted in the tables. Verapamil (not associated with QRS prolongation) has an $IC_{50} : fC_{max}$ ratio of 115, whereas propafenone (a QRS prolonging Class I drug) has an $IC_{50} : fC_{max}$ ratio of 1.3.

The same ratios were calculated for all 98 drugs, and the data are presented graphically in Figure 2. The data in Figure 2 are divided into the three drug categories. For category 1 drugs, the $IC_{50} : fC_{max}$ ratios ranged from 1.3 to 57.4 (mean = 13, n = 15). The majority (87%) of the Class I drugs had an $IC_{50} : fC_{max}$ ratio of less than 30. For category 2 drugs, margins ranged from 2.1 to 800 (mean = 85, n = 27). When categories 1 and 2 were combined, the majority of drugs (67%) had $IC_{50} : fC_{max}$ ratios of less than 30-fold. For category 3 drugs, the margins ranged from 13 to 406 050 (mean = 16 193, n = 55). The majority of category 3 drugs (72%) had ratios greater than 100.

In order to further characterize the relationship between the fC_{max} and $hNa_v1.5$ channel IC_{50} values, we carried out two separate analyses. Initially, we conducted a crude statistical analysis by fitting a Gaussian distribution to the frequency plots for the fC_{max} and IC_{50} values for the 98 drugs tested in this study. The frequency/distribution curves are plotted in Figure 3A. The separation between the two distribution means is 109 ± 1. It is worth noting that the distribution for the $hNa_v1.5$ IC_{50} values is quite narrow when compared to the drug fC_{max} values.

The data were then pooled into two groups: Class I antiarrhythmics and other drugs associated with QRS complex prolongation were classified as positives, and drugs not

Table 1

Class I anti-arrhythmics

Drug	MW	PPB %	Plasma concentration range (nM)	$hNa_v1.5\ IC_{50}$ (μM)	$Log_{10}\ IC_{50} : fC_{max}$	[QRS]free (nM)	Log_{10} mean IC_{50} : [QRS]free
Ajmaline	326.4	49	65–17 103	43.2	0.48	24 510	0.2
Aprindine	322.5	90	239–620	1.1	0.23	211; 192	0.7
Cibenzoline	262.4	55	976–1837	10.4	0.75	900	1.1
Disopyramide	339.5	69	742–6470	302.3	1.7	1950; 3327; 1950; 1756	2.1
Encainide	352.5	75	213–816	11.2	1.1	139; 202; 171; 348	1.7
Flecainide	414.3	57	753–840	5.8	0.84	265; 525; 559; 229	1.2
Lidocaine	234.3	67	7112–12 802	30.9	0.40	2077; 1963; 4552	1.1
Lorcainide	370.9	85	162	2.3	1.2	227; 113; 120	1.2
Mexiletine	179.3	67	1869–4129	25.7	0.79	1121; 9718	0.9
Moricizine	427.5	88	281	1.2	0.64	253; 393	0.6
Phenytoin	252.3	87	4360–7631	120.6	1.2		
Pilsicainide	272.4	27	2687	51.1	1.3	1290; 1451; 6503; 16 661	1.1
Procainamide	235.3	16	30 340–54 186	2050.0	1.8	35 695; 53 185; 26 771; 56 397; 29 270	1.7
Propafenone	341.5	90	241–879	1.2	0.13	88; 29; 1171; 81; 291	0.9
Quinidine	324.4	87	1566–3237	9.7	0.48	916; 1018; 1174; 1057	1.0

MW, molecular weight; PPB %, human plasma protein binding; [QRS]free (nM), the free plasma concentration associated with QRS complex prolongation in human – more than one value may be quoted, see supplemental data for more details. Log_{10} mean IC_{50} : [QRS] = log_{10} of ($hNa_v1.5\ IC_{50}$ divided by [QRS]free (nM). Plasma concentration range is the free (unbound) value. See supplemental tables for additional information and references.

Table 2

Drugs associated with QRS complex prolongation

Drug	MW	PPB %	Plasma concentration range (nM)	$hNa_v1.5$ IC_{50} (μM)	Log_{10} IC_{50} : fC_{max}	[QRS]free (nM)	Log_{10} mean IC_{50} : [QRS]free
Amiodarone	645.3	98	0.5–62	4.8	1.9	121; 47; 62; 39	1.9
Amitriptyline	277.4	95	4.9–49	1.6	1.5	208; 160	1.2
Amodiaquine	355.9	95	25–58	9.7	2.2	58	2.2
Amoxapine	313.8	90	16–191	5.8	1.5	287	1.3
Bupivacaine	288.4	96	32–312	4.3	1.1	190; 328	1.2
Bupropion	239.7	84	67–95	76.2	2.9	659	2.1
Carbamazepine	236.3	74	7061–13 034	152.0	1.1	57 493; 52 617	0.4
Chloroquine	319.9	58	660	36.5	1.7	16 900; 3848	0.7
Citalopram	324.4	80	46–123	14.7	2.1	1196	1.1
Desethylamodiaquine	327.8	86	353	6.9	0.44		
Desipramine	266.4	81	27–357	2.4	0.82	123	1.3
Diltiazem	414.5	86	1091	14.2	1.1		
Diphenhydramine	255.4	92	23–34	16.4	2.7	776	1.3
Dolasetron	324.4	72	637	79.7	2.1		
Fluoxetine	309.3	94	6.5–93	4.9	1.7	167	1.5
Imipramine	280.4	90	73–128	3.6	1.4	43; 74; 53	1.8
Lamotrigine	256.1	56	14 249–24 327	63.4	0.42	25 721	0.3
Maprotiline	277.4	89	10–238	2.8	1.1	83	1.5
Mesoridazine	386.6	95	129	7.2	1.7	2069	0.5
Nortriptyline	263.4	94	23–36	2.0	1.7	23	1.9
Perhexiline	277.5	90	216	3.4	1.2		
Procaine	236.3	6	39 778–39 778	84.6	0.33	40 574	0.3
Quinine	324.4	92	1834	17.8	1.0	2682; 995; 1336	1.1
Risperidone	410.5	89	2–2130	20.2	1.0	287	1.8
Ropivacaine	274.4	94	394	11.2	1.5	481	1.4
Thioridazine	370.6	98	84–979	3.5	0.55	262	1.1
Venlafaxine	277.4	27	395–1053	90.1	1.9	14 473	0.8

MW, molecular weight; PPB %, human plasma protein binding; [QRS]free (nM), the free plasma concentration associated with QRS complex prolongation in human – more than one value may be quoted, see supplemental data for more details. Log_{10} mean IC_{50} : [QRS] = Log_{10} of (hNav1.5 IC_{50} divided by [QRS]free) (nM). Plasma concentration range is the free (unbound) value. See supplemental tables for additional information and references.

Table 3

Drugs not associated with QRS complex prolongation

Drug	MW	PPB %	Plasma concentration range (nM)	$hNa_v1.5\ IC_{50}$ (μM)	$Log_{10}\ IC_{50} : fC_{max}$
Ambroxol	378.1	90	23	15.2	2.8
Astemizole	458.6	97	0.04–3.5	2.3	2.8
Bepridil HCl	366.6	100	5.1–33	1.9	1.8
Bromopride	344.3	40	105	41.4	2.6
Buspirone	385.5	91	0.9–1.0	125.4	5.1
Chloramphenicol	323.1	51	150 48–27 689	215.0	1.1
Chlorpromazine	318.9	96	25–65	2.8	1.6
Cinnarizine	368.5	91	67	1.0	1.2
Cisapride	466.0	0	4.9	4.2	2.9
Clemastine	343.9	N/A	4.7	4.0	2.9
Clomipramine	314.9	98	16–30	2.6	1.9
Clozapine	326.8	95	49–92	11.6	2.1
Cyproheptadine	287.4	96	6.0–7.0	6.1	2.9
Desloratadine	310.8	85	1.9	14.1	3.9
Diazepam	284.8	98	29–180	73.2	2.6
Dipyridamole	504.6	99	26–34	19.3	2.7
Domperidone	425.9	92	3.8–19	5.6	2.5
Ebastine	469.7	0	5.1	1.3	2.4
Exemestane	296.4	90	2.7	71.3	4.4
Fenfluramine	231.3	36	76–166	47.0	2.5
Flunarizine	404.5	93	5.5–34	2.6	1.9
Fluphenazine	437.5	90	0.5–0.9	8.0	3.9
Haloperidol	375.9	91	3.6–5.0	2.3	2.7
Hydroxyzine	374.9	93	19–22	32.8	3.2
Ketoconazole	531.4	99	113–709	44.9	1.8
Lofexidine	259.1	85	984	27.2	1.4
Loperamide	477.1	97	0.15–0.2	2.9	4.1
Loratadine	382.9	98	1.0–1.4	33.6	4.4
Memantine	179.3	45	256	92.6	2.6
Mepivacaine	246.4	77	373–817	81.4	2.0
Mifepristone	429.6	98	50–92	25.8	2.4
Moxifloxacin	401.4	42	6083	206.7	1.5
Nicardipine	479.5	99	2.3–2.6	4.3	3.2
Nifedipine	346.3	97	7.7–10	33.5	3.5
Nilvadipine	385.4	98	0.2–0.6	2.3	3.6
Olanzapine	312.4	93	1.8–6.7	39.0	3.8
Oxatomide	426.6	91	7.4	1.0	2.1
Pimozide	461.6	99	0.2–0.4	1.7	3.6
Prilocaine HCl	220.3	29	974–6491	72.6	1.9
Propranolol	259.4	93	32–80	7.5	2.0
Protriptyline	263.4	92	91	3.1	1.5
Pyrimethamine	248.7	86	154	24.6	2.2
Quinacrine	400.0	80	247	20.2	1.9
Reserpine	608.7	96	0.1	10.5	4.9
Riluzole	234.2	96	66–256	17.6	1.8

Table 3

Continued

Drug	MW	PPB %	Plasma concentration range (nM)	$hNa_v1.5$ IC_{50} (μM)	Log_{10} IC_{50} : fC_{max}
Salmeterol	415.6	96	0.14–0.1	7.2	4.7
Sertindole	441.0	99	1.6–1600	8.1	5.6
Sertraline	306.2	98	9.3–33	4.3	2.1
Tacrine	198.3	55	23–36	47.1	3.1
Terfenadine	471.7	97	0.3–0.6	2.0	3.5
Terguride (S–)	340.5	70	2.0	22.1	4.0
Trifluoperazine	407.5	99	0.04–0.2	7.4	4.5
Trimipramine	294.4	95	4.8–42	2.7	1.8
Verapamil	454.6	95	11–81	9.3	2.1
Ziprasidone	412.9	99	1.5–2.2	170.0	4.9

MW, molecular weight; PPB %, human plasma protein binding. Plasma concentration range is the free (unbound) value. See supplemental tables for additional information and references.

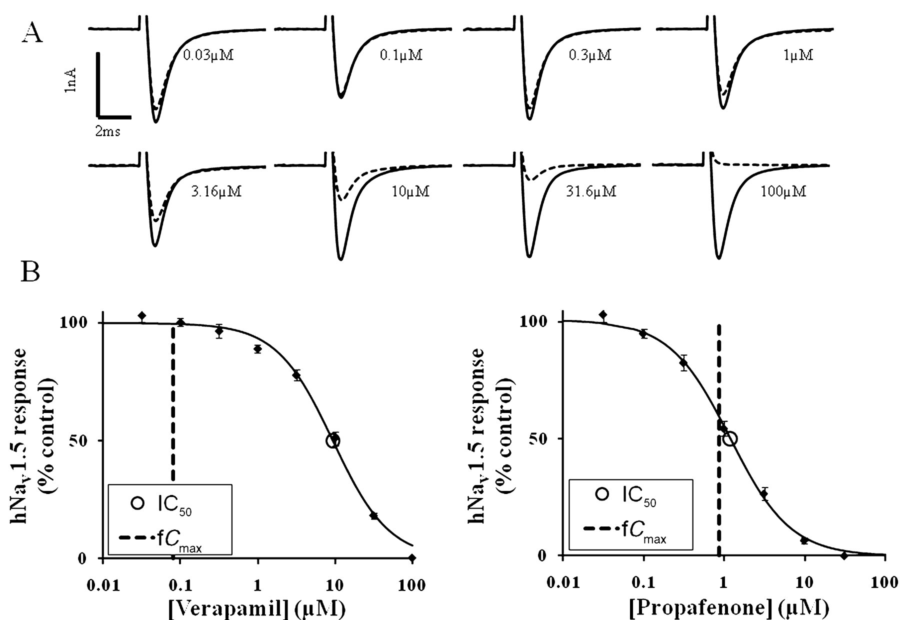

Figure 1

IC_{50} : fC_{max} ratio calculations for verapamil and propafenone. (A) Typical $hNa_v1.5$ current recordings generated using an IonWorks™-based assay. Each individual recording shows data from a single well in response to vehicle (0.33% dimethyl sulphoxide, solid line) followed by a single concentration of verapamil (dotted line). Each recording is taken from the eighth pulse to 0 mV from a holding potential of –90 mV. (B) Graphs show concentration–effect curves for $hNa_v1.5$ channels generated using the same assay. The half maximal inhibitory concentration (IC_{50}) and maximal free plasma concentration in human (fC_{max}) values are also plotted. For propafenone, the $hNa_v1.5$ IC_{50} is 1.2 μM and fC_{max} is 0.9 μM, giving a ratio (safety margin) of 1.3. For verapamil, the $hNa_v1.5$ IC_{50} is 9.3 μM and the fC_{max} is 0.08 μM, giving a ratio of 115.

reported to prolong the QRS complex were classified as negatives. The false positive/false negative incidence was then calculated for a range of IC_{50} : fC_{max} ratios [shown in Figure 3B as log_{10} (ratio)]. This analysis was conducted using both total and unbound (free) C_{max} values. The results of this analysis are plotted in Figure 3B. When only the free drug concentrations were taken into account, the point where the false positive/false negative incidence was equal occurred within the 30 to 100 ratio range. When total concentrations were taken into account, this point occurred between ratios of 1 and 10 [log_{10} (ratio) 0 to 1].

Finally, we attempted to determine the relationship between the $hNa_v1.5$ IC_{50} value and the free drug concentration that is associated with prolongation of the QRS complex

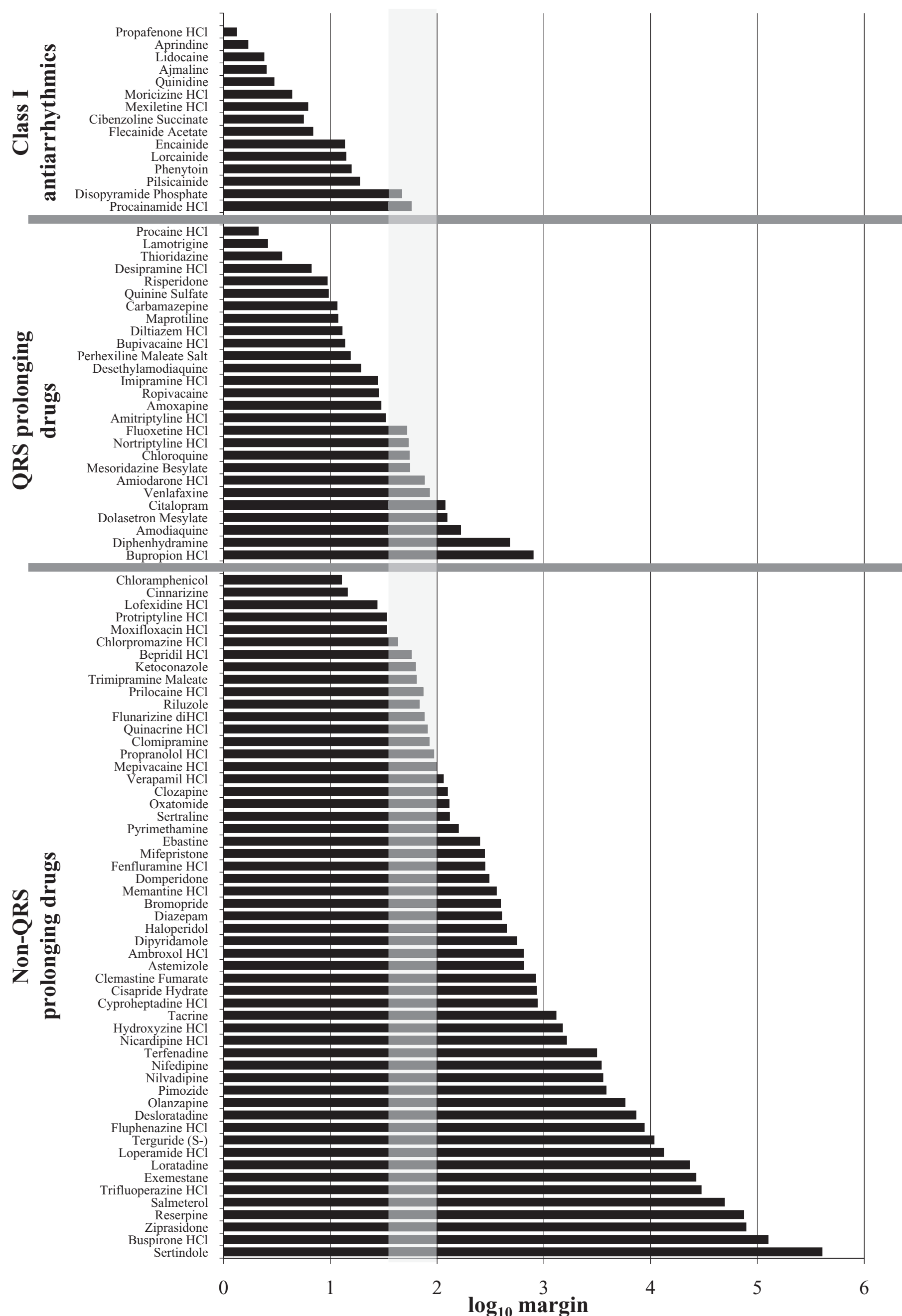

Figure 2

Log_{10} of the IC_{50} : fC_{max} ratios. IC_{50} values at $hNa_v1.5$ channels were generated using a IonWorks™-based assay. The fC_{max} values are derived from published values of the maximum plasma concentration achieved in a clinical setting and the plasma protein binding value in humans. The vertical grey box indicates where ratios between 30 and 100 would fall. For source data, refer to Tables 1–3.

in humans ([QRS]free). The IC_{50} : [QRS]free ratio (therapeutic window) was calculated for each drug where data were available. The results of this analysis for 37 drugs are presented in Figure 4. The IC_{50} : [QRS]free therapeutic window for these drugs ranged from 1.8 to 165 (mean = 15). It should be noted that for some drugs, the QRS data were derived from case studies that often involved drug overdose. Also, as noted previously, there is a paucity of drug-induced QRS clinical data in the literature on which to conduct this kind of analysis.

Discussion

The importance of preclinical assessment of blocking activity at $hNa_v1.5$ channels

Drug block of the cardiac Na^+ channel can be associated with intracardiac conduction delay, which in turn is manifested as prolongation of the QRS complex on the ECG (Delk *et al.*, 2007). Furthermore, ventricular tachycardia and other

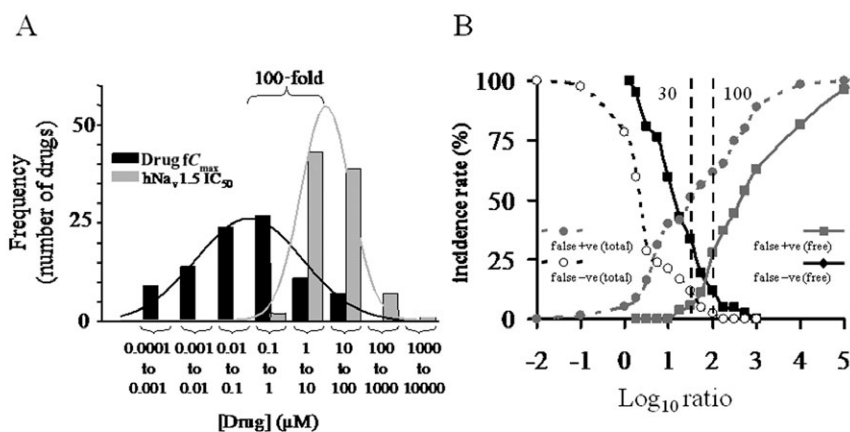

Figure 3

Analysis of fC_{max}, $hNa_v1.5$ IC_{50} and derived ratios. (A) Distribution curves for fC_{max} and IC_{50} values for the drugs analysed in this study. The distribution means [expressed as $\log_{10}$ of (drug)] were –6.6 ± 0.1 (R^2 = 0.95) for fC_{max} data and –4.5 ± 0.03 (R^2 = 0.99) for the IC_{50} data. (B) Analysis of false positive (false +ve) and false negative (false –ve) rates versus IC_{50} : fC_{max} ratios. Class I anti-arrhythmics (Na^+ channel blockers) and other known QRS-prolonging drugs were classified as positive, and drugs not known to prolong QRS were classified as negative. The false positive/negative incidence was calculated for ratios using total and unbound (free) C_{max} values. A ratio of 30–100 has the optimal false positive/negative incidence for free drug concentration, as indicated by the dotted line.

arrhythmias are considered to contribute to the morbidity and mortality associated with Na^+ channel blocking drugs (Thanacoody and Thomas, 2005; Delk *et al*., 2007). However, Seger (2006) has suggested that the true incidence and aetiology of ventricular arrhythmias is unknown: 'There is no evidence that the arrhythmias that occur in (Na^+ channel blocking drug) poisoning prior to the development of a failing heart are a result of non-uniform conduction slowing.' Despite this, a drug-induced change to the ECG, such as QRS prolongation, remains an undesirable property for new chemical entities. For example, QRS prolongation has been identified as a marker for increased mortality (Horwich *et al*., 2003; Kalahasti *et al*., 2003; Breidthardt *et al*., 2007; Adesanya *et al*., 2008). Therefore, it seems prudent to screen out liability to block $hNa_v1.5$ channels early in a drug discovery programme, where a medium-throughput $hNa_v1.5$ channel assay could generate data in a time frame that could influence medicinal chemistry design–make–test cycle (Harmer *et al*., 2008).

Pharmaceutical regulators have also highlighted the importance of drug-induced ECG changes – it is a requirement to perform a thorough ECG assessment before a candidate drug enters the final stage of clinical development (ICH E14 guidelines: Anonymous, 2005a). The primary aim of the thorough ECG assessment is to determine the effects of the candidate drug on the QTc interval as a surrogate marker for the cardiac arrhythmia TdP. Although QTc prolongation is recognized as an imperfect marker for TdP risk, the potential for sudden death and the difficulty in predicting such a rare arrhythmia has resulted in such prolongation becoming a major focus for drug safety (Darpo, 2010). If we have learnt anything from the hERG/QT/TdP story, it is that the best way to minimize uncertainty over the link between QT prolongation and TdP is to avoid having QT interval prolongation in the first place, by early application of the hERG screen (Pollard *et al*., 2010; Valentin *et al*., 2010).

An analogous situation exists for block of $hNa_v1.5$ channels and drug-induced QRS changes: the relationship between QRS prolongation and arrhythmia is poorly defined. Nonetheless, given the choice between two otherwise equal new drugs, one that does not widen QRS and one that does, the former would be the rational choice. Furthermore, given the association between QRS prolongation and mortality, and the potential for drug-induced arrhythmia, it remains a sensible precaution to remove any activity at $hNa_v1.5$ channels before compounds reach human volunteers.

In this study, we have avoided making any link between potency as a blocker of $hNa_v1.5$ channels and arrhythmia risk, as this is a challenging task beyond the scope of this study. However, it is worth pointing out that preclinical cardiac risk assessment would also include integrating information from other ion channel assays (hERG, L-type Ca^{2+} channel, etc.), as well as other *in vitro* and *in vivo* cardiovascular assays. Taken together, the integrated data would give an assessment of the risk of a candidate drug causing an ECG change in humans. However, such data are not designed to assess the pro-arrhythmic risk *per se*. Ultimately, the only way to determine if a drug has pro-arrhythmic potential is to conduct a large and expensive longitudinal trial in patients to assess cardiovascular risk. Therefore, screening out any liability to interact with cardiac ion channels early in the drug discovery process is the simplest route to reduce potential cardiac safety issues.

Safety margins for compounds with activity at $hNa_v1.5$ channels

Given the importance of preclinical screening on $hNa_v1.5$ channels, the interpretation of such data should also be given serious consideration – that is, how does a drug discovery programme place the IC_{50} at $hNa_v1.5$ channels in context? To address this question, we set out to define what should be considered a safe margin between a $hNa_v1.5$ IC_{50} and the free

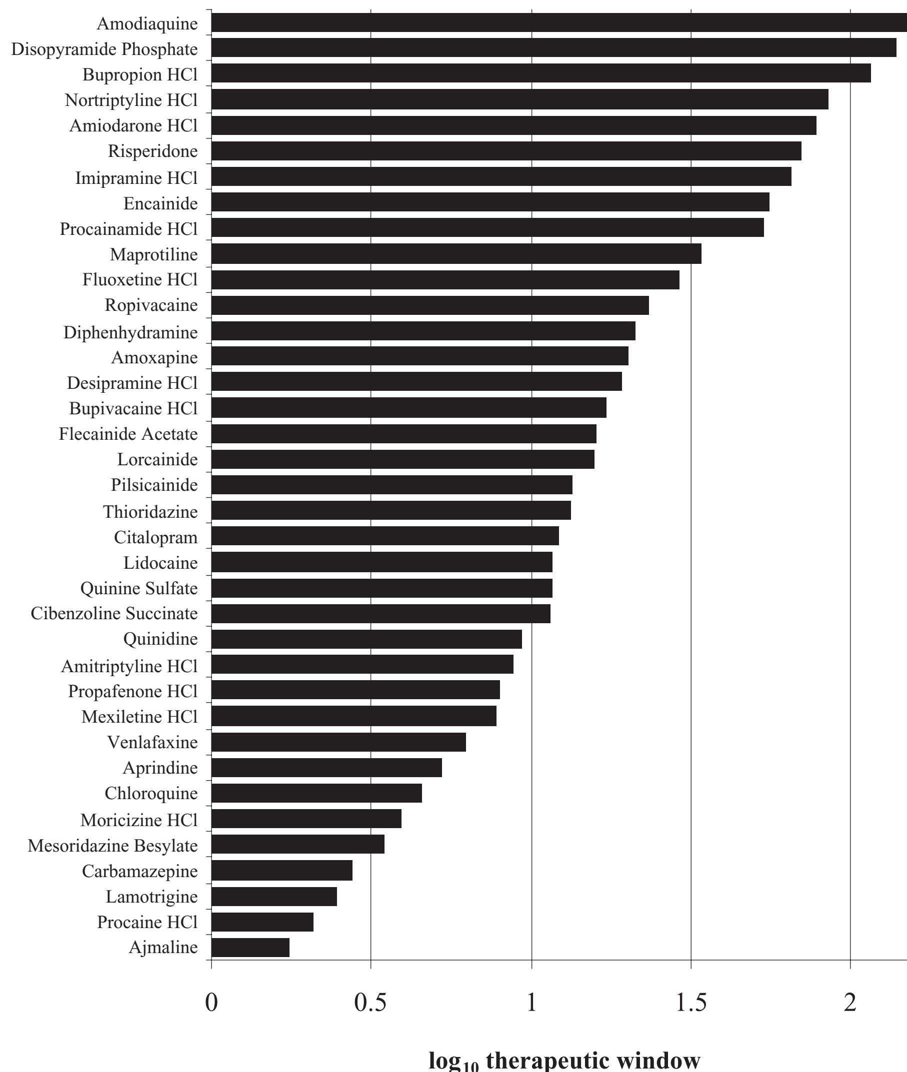

Figure 4

Log_{10} of ($hNa_v1.5$ IC_{50} : [QRS]free), shown as therapeutic window. IC_{50} data were generated using the IonWorks™-based assay. [QRS]free is the published value of mean lowest free plasma concentration associated with prolongation of the QRS complex in humans. For source data, refer to Tables 1 and 2.

drug concentration that is achieved in man. A similar approach has been taken by several authors for the hERG K^+ channel (Webster *et al.*, 2002; Redfern *et al.*, 2003).

The data presented here indicate that a ratio of 30–100 between $hNa_v1.5$ IC_{50} and fC_{max} would be sufficient to ensure a suitable degree of safety in terms of drug-induced QRS complex prolongation. This 30–100 ratio represents the optimal range over which false positives and false negatives are at a minimum (see Figure 3B). A number of investigators have come to a similar conclusion regarding safety margins applied to blockade of hERG channels (Webster *et al.*, 2002; Redfern *et al.*, 2003).

We would recommend this safety margin to drug discovery projects that have liability to block $hNa_v1.5$ channels and a reliable estimate of the fC_{max} in human. However, this ratio should only be regarded as an initial guide – that is, the IC_{50} at $hNa_v1.5$ channels within a 30- to 100-fold margin should act as a safety flag. Once *in vivo* ECG data are available, this should also be taken into account when drawing up an integrated risk assessment (Valentin and Hammond, 2008). In addition to this, the indication for which the drug is intended should be considered. For example, a 10-fold safety margin may be warranted for life-threatening indications (e.g. late-stage cancers or patients with severe hospitalized infections) whereas a 100-fold safety margin would be more appropriate for indications such as eczema or seasonal rhinitis. The safety profile of existing therapies is also important – a drug that does not affect the QRS complex might be favoured over one that does have this liability. Other factors that also influence the risk assessment include the duration of treatment and the likely patient population (for instance, do patients have underlying cardiac disease?).

Relationship between IC_{50} at $hNa_v1.5$ channels and plasma concentrations evoking a QRS change

We wanted to understand how $hNa_v1.5$ IC_{50} data compared with the free plasma concentration that evokes a QRS change in man ([QRS]free), which we have called the therapeutic window). For the 37 drugs for which we could obtain human pharmacodynamic and pharmacokinetic data, there was a wide range in this therapeutic window. Nonetheless, these data suggest that, on average, a QRS change can be evoked by a free plasma concentration 15-fold less than the corresponding IC_{50} for $hNa_v1.5$ channels – that is, QRS complex prolongation can occur to the left of the $hNa_v1.5$ IC_{50}. A preliminary analysis, based on a limited number of compounds, by Cordes *et al*. (2009) has come to a similar conclusion. These analyses suggest that <10% inhibition of the $hNa_v1.5$ channel could be associated with prolongation of the QRS complex in man.

The reasons why there is such a wide range in the IC_{50} : [QRS] therapeutic window are likely to be diverse. One possible explanation could include the varying quality of the clinical data. For some drugs, the QRS data come from patients who have taken a drug overdose, or from patients with underlying cardiac disease, whereas other drug data come from controlled clinical trials. Secondly, in some cases, measurement of blood plasma levels and QRS intervals may not be optimal. For example, plasma samples may only have been collected as a single time point during dosing, while manual measurement of the QRS complex duration can also be prone to error. Finally, the kinetics with which drugs bind to the $hNa_v1.5$ channel may also influence the therapeutic window. Drugs with rapid on/off kinetics may require high plasma concentrations to affect the duration of the QRS complex duration – and this may only occur at high heart rates (Vaughan Williams, 1991; Takanaka *et al*., 1994).

Outlying drugs

Analysis of the calculated ratios has identified a number of outliers, that is, drugs where the $IC_{50} : fC_{max}$ ratio is not what is expected for that category. For example, outliers in the Class I antiarrhythmic group are disopyramide and procainamide, with an $IC_{50} : fC_{max}$ ratio of 47 and 57, respectively. Other drugs in the same category tend to have ratios below 30. The reason for this discrepancy is not clear. A plausible explanation is that the $hNa_v1.5$ assay employed in this study underestimated the potency of these compounds (see *Limitations of this study*). Yatani and Akaike (1985) obtained an IC_{50} for disopyramide of 28 μM, determined using the Na^+ current measured in isolated cardiac myocytes. This value is 10-fold less than the IC_{50} value determined using the IonWorks $hNa_v1.5$ assay (302 μM) and would lead to an $IC_{50} : fC_{max}$ ratio of 4.3, which is in line with the ratios for other Class I anti-arrhythmics.

Amodiaquine is an outlier in category 2, with a margin of 166. However, its main metabolite, desethylamodiaquine, has a margin of only 19. Both parent and metabolite molecules are approximately equipotent at $hNa_v1.5$ channels, although the metabolite has a sixfold higher free plasma concentration. It is plausible that other drugs have metabolites with either higher plasma concentrations or higher potencies at $hNa_v1.5$ channels. Screening these metabolites in the $hNa_v1.5$ channel assay would test this hypothesis.

Some of the outliers in category 3, such as chloramphenicol and cinnarizine, have lower ratios compared with the category as a whole. Given these low ratios, one would expect these drugs to cause QRS prolongation in a clinical setting. It is possible that the fC_{max} data for these drugs are not reflective of plasma concentrations seen in normal clinical practice (e.g. chloramphenicol is mainly used as a topical eye treatment in developed countries). Alternatively, measurements of QRS complex prolongation may have been under-reported for patients taking these drugs.

A plausible explanation is not obvious for many of the other outlying drugs. However, one possibility is differential accumulation of the compound in the myocardium – a phenomenon recognized for a number of drugs (Jensen and Nielsen-Kudsk, 1988; Yoshida and Furuta, 1999; Titier *et al*., 2004). In addition, impulse conduction in the heart is determined by three factors: (i) cellular excitability (e.g. $hNa_v1.5$ channel activity); (ii) electrical coupling (e.g. connexin43 in the ventricle); and (iii) cellular/tissue architecture (e.g. fibrosis, myocyte size and shape). If a compound affects any of these parameters, then it could also potentially affect QRS duration.

It should be noted that it was not possible to obtain IC_{50} values for 34 drugs as these were either inactive or only partially active at $hNa_v1.5$ channels. Of these 34 drugs, three are associated with QRS complex prolongation in man (amantadine, atenolol and sotalol), placing them into category 2 (Freedman *et al*., 1992; Schwartz *et al*., 2008). These drugs caused 25% to 30% inhibition in the $hNa_v1.5$ channel assay. The remaining 31 drugs are not associated with QRS complex prolongation in man, which places them in category 3.

Limitations of this study

There are a number of considerations that need to be taken into account that limit the interpretation of this study. The primary limitation is the lack of clinical data where drug-induced QRS changes have been accurately measured, and pharmacokinetic data have been simultaneously determined. This makes it difficult to estimate the therapeutic window between IC_{50} at $hNa_v1.5$ channels and the concentration causing a QRS change. Much of the data relating to drug-induced QRS effects are derived from isolated case studies (including drug overdose) and from clinical trials conducted on patients with underlying cardiac disease. Clearly, use of such data is sub-optimal, but until more comprehensive clinical data are available, no other analysis is possible. It is our hope that more comprehensive pharmacokinetic/pharmacodynamic data relating to drug-induced QRS changes in man will emerge as the importance of this cardiotoxicity receives more attention. When more comprehensive clinical data are available, we may also be able to determine if specific patient populations are more susceptible to QRS prolonging drugs (e.g. patients with ischaemic heart disease or those with impaired cardiac conduction).

It should also be noted that we could not take into account the magnitude of any drug-induced QRS complex prolongation, as these data were either unavailable or quite variable. For example, we have not differentiated between

marked increases in QRS complex duration seen with some Class I anti-arrhythmics (e.g. the ~30% increase seen with encainide) and small QRS complex changes seen with some other drugs (e.g. amodiaquine). The magnitude of QRS complex prolongation is likely to be important, as evidence from patients with right bundle branch block indicates that mortality increases in line with prolongation of the QRS complex (Adesanya *et al.*, 2008). Until more detailed clinical data are available, it remains difficult to quantify what constitutes a 'safe' degree of QRS complex prolongation.

Another limitation is the reliance upon C_{max} and human plasma protein binding data. During the compilation of these data, it was apparent that some drugs have a wide range of reported therapeutic plasma concentration values (e.g. 65 to 17 103 nM for ajmaline). Furthermore, it was also obvious that the fC_{max} calculation was dependent upon the plasma protein binding data. Clearly, for the ratio used here, an inaccurate fC_{max} value will return an equally inaccurate ratio. However, it is our contention that although these variables do add a certain amount of 'noise' to the data, they do not affect the overall conclusions reached.

We have chosen to use the IC_{50} at $hNa_v1.5$ channels, as this is the most accurate measurement that can be made from a sigmoidal log_{10} concentration–effect curve. An alternative approach would have been to use IC_{10} or IC_{20} values. The latter method was excluded on the grounds that it relies upon setting a smaller margin based on an intercept derived from an unreliable part of the sigmoidal log_{10} concentration–effect curve, barely above the background noise. However, for compounds that inhibit $hNa_v1.5$ channels by less that 50% (and thus do not have an IC_{50} value), the use of IC_{10} or IC_{20} may be of some value.

The basis of this paper was to describe the $IC_{50} : fC_{max}$ ratios for a range of drugs. We addressed this challenge by separating the drugs into three categories (Class I anti-arrhythmics, other known QRS prolonging drugs, drugs not known to prolong QRS). However, a better approach would have been to stratify these drugs into groups of varying incidence of causing QRS prolongation (i.e. high, low or no QRS prolongation). Unfortunately, these data are not currently available in the literature.

In this study we employed a single assay to determine blocking potencies at $hNa_v1.5$ channels. This has the advantage of a single data set generated using a robust and reliable method, with minimal experimental variation. This contrasts with previous studies, which examined the relationship between hERG IC_{50} and fC_{max} – these employed hERG IC_{50} values quoted in the literature (Webster *et al.*, 2002; Redfern *et al.*, 2003). Nonetheless, the IonWorks™ $hNa_v1.5$ channel assay does have the potential to generate variable data in the following respects. Firstly, the assay has a defined experimental design (i.e. $hNa_v1.5$ currents are generated at 3 Hz, and compounds are incubated for a fixed 3 min period). It is therefore possible that some compounds may not have reached steady-state inhibition of the $hNa_v1.5$ channel, resulting in underestimates of the IC_{50}. Secondly, the $hNa_v1.5$ assay has a false negative rate of 8% when compared to assay of the effects on the upstroke of the canine Purkinje fibres action potential (Harmer *et al.*, 2008). This raises the possibility that some compounds may cause more pronounced block of the cardiac Na^+ channel when measured using native systems. Finally, although planar patch clamp recordings are considered fit for purpose within a screening context, the data generated using this method do not always correlate with that generated using conventional electrophysiology. For example, lipophilic compounds can adsorb non-specifically to plastic surfaces in the automated systems, potentially causing a rightward shift in compound IC_{50} values (Dunlop *et al.*, 2008).

A subject that is often raised when considering safety margins is the use of total versus free plasma C_{max} values. Drug that is bound to protein is not able to interact with ion channels on the cell surface. Therefore, it seems intuitive to use free drug concentrations. Indeed, several other studies have successfully adopted the free-drug approach when comparing *in vitro* and clinical data for the purposes of cardiac risk assessment (Kang *et al.*, 2001; Webster *et al.*, 2002; Redfern *et al.*, 2003). The data presented here indicate that the use of total plasma concentrations is no better than free concentrations (see Figure 3B). We have therefore focused on setting $hNa_v1.5$ IC_{50} safety margins based on free plasma concentrations given the clear scientific rationale for using this approach.

Despite the clear limitations of this study, we feel that the data are of sufficient value to support the conclusion reached in this paper.

Conclusions

The principal aim of this study was to generate data that would provide evidence for setting provisional safety margins, and aid in the interpretation of potency data for blockade of $hNa_v1.5$ channels, obtained during preclinical safety screening. Based on the data presented here, we have concluded that a safety margin of 30- to 100-fold should be adopted early in the drug discovery process, where no other supporting data are available. However, inhibition of $hNa_v1.5$ channels should only be considered as a safety flag – an integrated preclinical assessment of all *in vitro* and *in vivo* cardiovascular data is essential in order to fully understand the risk/benefit of a compound, prior to progression into human trials.

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Conflict of interest

None declared.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Class I anti-arrhythmics
Table S3 Drugs associated with QRS complex prolongation
Table S3 Drugs not associated with QRS complex prolongation

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