Editorial

Innovation in safety pharmacology testing

M.K. Pugsley a,⁎, R. Towart b, S. Authier c,d, D.J. Gallacher b, M.J. Curtis e

a Department of Toxicology/Pathology, Johnson & Johnson PRD, Raritan, NJ, USA
b Center of Excellence for Cardiovascular Safety and Mechanistic Pharmacology, Johnson & Johnson Pharmaceutical Research and Development, Division of Janssen Pharmaceutica NV, Turnhoutseweg 30, B-2340 Beerse, Belgium
c Faculté de médecine vétérinaire, Université de Montréal, P.O. Box 5000, St-Hyacinthe, QC, Canada J2S 7C6
d LAB Research Inc., 445 Armand Frappier, Laval, QC, Canada H7V 4B3
e Cardiovascular Division, King’s College London, Rayne Institute, St Thomas’ Hospital, London SE17 7BQ, UK

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This issue of the Journal of Pharmacological and Toxicological Methods (JPTM) is themed. It is the eighth in a series, arising from the Annual Safety Pharmacology Society (SPS) meeting. The SPS is now in its 10th year as an independent branch of biological sciences (distinct from pharmacology and toxicology) and is the primary forum for driving advances in safety pharmacology. The theme of the meeting and this journal issue is innovation, and the focus is non-clinical safety assessment of new chemical entity (NCEs). The content is informed by regulatory guidance documents (S7A and S7B) prior to first in human (FIH) studies. The manuscripts cover a broad spectrum of safety pharmacology topics from theory to practice, with interrogation of state-of-the-art techniques, and profiling of methods that are in development for safety assessment. Philosophical and strategic issues are addressed, with consideration of the use of novel methods for population pharmacokinetic (PK) analysis, abuse liability, electrocardiogram (ECG) analysis algorithms, in vitro cardiac slice preparations, human pluripotent stem cells, and a brief discussion regarding the assessment of changes in the QRS complex of the ECG indicative of drug-induced blockade of cardiac sodium channels. Safety pharmacology methods continue to evolve.

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1. Introduction

This issue of JPTM focuses exclusively on safety pharmacology (defined as those studies that investigate the potential undesirable pharmacodynamic effects of a substance on vital physiological functions in relation to exposure in the therapeutic range and above) and associated non-clinical methods (Bass, Kinter, & Williams, 2004; Pugsley, Authier, & Curtis, 2008; U.S. Food & Drug Administration, 2001). This is the 8th consecutive year the journal has published a themed issue on this fundamental topic for the Pharmaceutical Industry. The issue interrogates methods and models that have been developed specifically for the assessment of the safety profile of NCEs, as required according to ICH guidelines (see S7A and S7B for details), prior to conduct of FIH studies (U.S. Food & Drug Administration, 2001, 2005). The manuscripts that follow derive from presentations made at the recent Safety Pharmacology Society meeting in Boston, MA (for a comprehensive overview of the meeting, see Cavero, 2011). The Society is now in its 10th year, and it defines and shapes safety pharmacology as an independent branch of biological sciences (distinct from pharmacology and toxicology).

The manuscripts herein cover a broad spectrum of safety pharmacology topics. In some articles, discussion of important issues concerning the current regulatory environment is included. The manuscripts in this issue of JPTM also highlight novel areas where safety pharmacology may have greater impact in the future. We encourage further discussion of key issues, particularly consideration of the predictiveness of the non-clinical assays described.

2. Themes

2.1. Abuse liability testing

Abuse liability testing has emerged to the forefront of safety pharmacology in recent years due to the development of several guidelines and discussion documents from regulatory authorities in the US and Europe (Markgraf & Kallman, 2009; Moser, Wolinsky, Castagné, & Duxon, 2011). Increasing concern about prescription drug abuse and the withdrawal potential of many common drugs have prompted development of non-clinical and clinical evaluation procedures (Moser, Wolinsky, Castagné, et al., 2011). A need for such assessment is made clear, particularly for all new CNS-active medicinal products (parents + metabolites) that can cross the blood

⁎ Corresponding author at: Department of Toxicology/Pathology, Johnson & Johnson PRD, OMP 2273, 1000 Route 202 South, Raritan, NJ 08869, USA. Tel.: +1 908 704 4148; fax: +1 908 635 3977.
E-mail address: mpugsley2@its.jnj.com (M.K. Pugsley).
brain barrier (BBB), interact with central targets and precipitate an abuse liability potential. Thus, non-clinical testing should result in data derived from a robust, fundamentally sound set of studies that provides for a complete assessment of drug abuse potential for a NCE.

Abuse liability potential is assessed from information obtained by two-tier evaluation (European Medicines Agency, 2006, 2008; U.S. Food & Drug Administration, 2010). The first tier evaluation compares the pharmacology of the NCE to drugs of known abuse potential. In vitro receptor binding studies determine if the NCE interacts with receptors associated with drug dependence (e.g., GABA, opioid and dopamine). Additional functional cellular assays (measuring neurotransmitter release and second messenger activity) may be conducted to determine the nature of the interaction (i.e., whether the drug is an agonist or antagonist). Thus, in vitro binding and functional cellular studies conducted as a part of early development can provide signals for possible dependence liability. If a signal is detected, a second tier in vivo animal behavior evaluation should be undertaken to directly assess the risk. The key issue here is the validation of the signal, and validation of the second tier assessments. The latter studies include drug discrimination (which determines the similarity of the subjective effects of a test drug to the subjective effects produced by a known drug of abuse), self-administration (which assesses the intrinsic rewarding properties of a drug), conditioned place preference (in which learned association between environmental stimuli and drug effect occurs), psychomotor tests (which assesses the effects of the test drug on motor function compared with drugs of abuse) and withdrawal (or discontinuation syndrome that occurs after abrupt drug cessation following chronic administration) (Aitor & Griffiths, 2003; Moser, Wolinsky, Castagné, et al., 2011; Moser, Wolinsky, Duxon, & Porsolt, 2011). Study findings should accord with clinical data and inform it, facilitating scheduling by regulators (U.S. Food & Drug Administration, 2010). Froger-Colléaux et al. (2011) show, for the first time, how implanted telemetry devices that continuously monitor vital function (body temperature, locomotor activity, heart rate and blood pressure) in conjunction with body weight and food intake increase the sensitivity and better define the time-course of withdrawal effects in rats. These authors showed that the intensity and duration of effects resulting from chronic morphine and chloridiazepoxide (given for 20 days of treatment and 8 days of withdrawal) administration were dose-related, suggesting that these methods may be useful in abuse liability evaluation. Further study in this area is warranted.

2.2. Frontloading CNS core battery studies

The Irwin test and functional observation battery (FOB) have a long history for use in assessment of CNS adverse effect liability (Porsolt, Lemaire, Durmuller, & Roux, 2002). This core battery study requirement is outlined in the ICH S7A guidance. As with other core battery assays, the relevant tests are usually conducted under GLP conditions and prior to FIH studies. However, early non-GLP CNS screening studies can be conducted in the drug discovery phase with lead compounds in order to gage the potential for safety issues early in development or to aid in selecting a lead candidate with reduced or absent CNS effects. In general, the study is conducted in a manner similar to the standard assay whereby the effects of the NCE are assessed according to different domains of CNS behaviors and for motor activity, reflexes, musculature, general excitation and seizure potential. Lynch, Castagné, Moser, and Mittelstadt (2011) examined spontaneous locomotor activity using two automated test systems: the Actimeter (Panlab/Harvard Apparatus) infrared photocell-based activity meter (with 15 levels of sensitivity in order to adapt to the topology of the animal, i.e., rat or mouse) and LABORAS (or Laboratory Animal Behavior Observation Registration and Analysis System, Metris B.V.) mechanical vibration and force-based signal analysis activity meter. Both systems determine movement and pattern of movement. Using caffeine and chlorpromazine in rats, the authors found that both systems exhibited similar sensitivities in determination of drug-induced changes in locomotor activity (Lynch et al., 2011). Thus, addition of automated systems could aid in ease of assessment in CNS adverse effect liability frontloading studies.

2.3. Safety biomarkers

A biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” (NIH Biomarkers Definitions Working Group, 2001). A simpler definition is a variable that is used as a surrogate for the variable of primary interest (Pugsley et al., 2008), e.g., QTc (prolongation) for torsades de pointes (TdP) liability. Biomarkers have always been a cornerstone in medical research. Simple biomarkers include specific proteins that can be quantified in the blood to detect the severity of a specific disease state. Established biomarkers include indices of cardiac or liver injury such as troponin-I or transaminases, respectively. Over the past few years biomarker development has evolved to the extent that their readout may be used to inform decision making in early drug development and safety assessment, under the assumption that biomarkers reflect a conceptual link between the underlying pathophysiological process and the disease state (Authier, Pugsley, Troncy, & Curtis, 2009). Thus, biomarkers serve a role in translational research, informing practical decision algorithms for clinicians, and informing early identification of the effectiveness and the safety of new therapies in pharmaceutical development. Biomarker use is therefore straightforward. However, a biomarker has no predictive reliability until it is validated.

Nodop Mazurek et al. (2011) address the issue of the use of rotarod performance, in conjunction with histological evidence of joint damage, to predict drug-induced musculoskeletal syndrome (MSS). MSS is characterized in humans by a host of signs and symptoms including inflammation, joint stiffness and localized pain, usually in the limbs. Clinically, MSS comprises individuals affected by fibromyalgia and myofascial pain syndrome but it may also be triggered as an adverse drug action, as was observed with matrix metalloproteinase (MMP) inhibitors, such as marimastat, used in oncology (Peterson, 2006). In marimastat-treated rats, reduced rotarod performance was found to accord with MSS-induced histopathology thus providing an in vivo functional biomarker with which to facilitate lead compound selection for drug discovery with MMP inhibitors. Biomarkers will occupy an emerging role in the strategy of drug discovery, and research that serves to interrogate their validity will underpin their emergence.

2.4. Stand alone vs. integrated study designs – future or past?

Safety pharmacology continues to make use of ‘stand alone’ (i.e., a study that utilizes a single study design for a single purpose) and ‘integrated’ (i.e., a study that utilizes a single modified design to assess complementary but distinctly different endpoints) approaches to study design (Pugsley, Authier, Towar, Gallagher, & Curtis, 2009). The present issue reflects this. Interlaboratory comparison is encouraged as a means to explore the validation of integrated designs.

The journal has in the past featured interrogation of integrated models. For example, Authier, Haefner, Fournier, Troncy, and Moon (2010) surgically implanted non-human primates (NHP) with telemetry transmitters to record arterial pressure and tidal volume in addition to standard CV safety study parameters (i.e., the ECG, physical activity and body temperature measurements), and Moscardo et al. (2010) modified a standard NHP cardiovascular safety assessment study design in order to include a neurobehavioral assessment. Both studies allow for
an integration of core battery study methods and evaluation of drug responses which may hold promise in the future.

In the current issue of JPTM, Authier, Gervais, Fournier, Gauvin, and Troncy (2011) examined the minipig as a potential safety pharmacology non-clinical animal species and evaluated the effects of many positive control drugs on respiratory and cardiovascular responses in an integrated study design. Readers are referred to a previous focused issue of JPTM (Nov–Dec 2010) that contains many manuscripts relating to the minipig RETHINK Project (see Curtis, 2010). The objective of RETHINK was to evaluate the potential value of toxicity testing in the minipig as an alternative approach in regulatory toxicity testing that advances the principles of replacement, refinement, and reduction, i.e., the 3R’s (Forster, Bode, Elleegaard, van der Laan, & Steering Group of the RETHINK Project, 2010). Briefly, the project assembled expert study groups to review multiple areas (ethics, welfare, and development of new medicines, safety testing and emerging technologies) related to the use of minipigs in regulatory safety testing. Authier et al. (2011) report that the minipig produces the required profile of responses to a range of drugs, and could be a valuable alternative non–rodent species for use in safety pharmacology studies. Use of the minipig is an interesting topic that will doubtless be revisited in the journal.

Thus, the integration of multiple core battery studies and the recording of multiple variables concurrently or consecutively in a single species streamline study design methods with the potential to reduce or even eliminate the need for parallel independent studies, thus adhering to the 3R principles for animal use in research (Balls, 1994). In addition to ethical and economical advantages, integrated study designs also enable assessment of vital system inter–dependency. NCE changes to the respiratory function may result from cardiovascular (CV) or CNS effects and vice versa. It is important to note that validation of integrated model designs requires consideration of the impact of the influence of the necessary method to derive a readout on the other readouts (e.g., the influence of CNS recording methods on CV readout, and vice versa). For the future, elaboration of integrated models may ultimately lead to the ability to assess all core battery endpoints in a single study design.

2.5. Cardiac sodium ion channel block — CAST out...or not?

The ECG is a critical diagnostic tool in safety assessment, and signal alterations are important biomarkers in non-clinical safety assessment (Authier et al., 2010). The ECG provides an overview of global electrophysiological function in the heart. Cardiac rhythm and the duration of the intervals including PR, QRS and QT represent the primary readout. Analysis of the amplitude, duration and morphology of the P-wave, QRS complex, ST-T, T-wave (and U-wave if detected) provides additional insight into the specific cardiac region affected by the NCE. The focus in recent years has been on ventricular repolarization and TdP, with the validity of surrogate endpoints especially QT interval prolongation and inward rectifying potassium current (I_K) blockade being the main preoccupation (Pugsley et al., 2008). However, attention has recently been drawn again to depolarization and conduction, targets that have relevance to proarrhythmia for drugs that block inward currents, particularly sodium current (I_{Na}). Drugs that slow ventricular conduction by I_{Na} blockade widen the QRS interval. There has been recent discussion about proposing ‘margins of safety’ for drug-induced sodium channel blockade (Harmer, Valentin, & Pollard, 2011). However, in order to appreciate the complexity of the issue and attempt to understand the implications of ascribing acceptable safety margins for tolerable drug-induced changes it is important to briefly review Class I antiarrhythmic sodium channel blocker drug history. Although I_{Na} may be regarded latterly as a target mediating adverse drug actions, it was regarded for decades as a target for therapeutic (ventricular antiarrhythmic) drug discovery until the notion was debunked by the Cardiac Arrhythmia Suppression Trial (CAST, Echt et al., 1991).

Our understanding of the nature of antiarrhythmic drugs (especially Class I sodium channel blockers) and their effects on cardiac electrical activity have markedly advanced since the observations by Hodgkin and Huxley (1952) of the electrical properties of the sodium current using the squid giant axon as a model. The result of this work provided the first implicit model for ion channel function whereby the sodium channel may exist in three states: rested (closed), activated (open), and inactivated (closed) and operate as a function of voltage and time and depend on membrane potential. Weidmann (1955) noticed that in the presence of drugs that block sodium channels (including agents such as quinidine, procainamide, and diphenhydramine) there were observed changes in the voltage-dependence of the maximum rate of depolarization (V_{max}) providing the fundamental tenets for the development of inward current targeted antiarrhythmic drug theory (Pugsley, 2002).

Drugs (i.e., Class I agents) that target I_{Na} were found to have activity in a range of animal models of arrhythmias, and initially showed efficacy in the clinic against non-life-threatening ventricular premature beats (VPBs). This resulted in their evaluation in larger Phase III clinical trials as prophylaxis against lethal VF under the assumption that efficacy against any ventricular arrhythmia predicted efficacy against any other (the ‘Cardiac Arrhythmia Suppression Hypothesis’). Subsequently, CAST, a large placebo-controlled study, was undertaken to examine whether the incidence of cardiac death in survivors of acute myocardial infarction (MI) with asymptomatic or mild ventricular arrhythmias, could be reduced with Class I sodium channel blocking antiarrhythmic drugs (Echt et al., 1991). The study examined flecainide, encainide and later moricizine (in CAST-II), and mexiletine was evaluated in the IMPACT (International Mexiletine and Placebo Antiarrhythmic Coronary Trial). The results were catastrophic, with an abnormally high incidence of death in drug treated patients compared with placebo controls; thus, further drug development was abandoned and as a result I_{Na} blocking antiarrhythmic drugs are not normally used today to suppress life-threatening ventricular arrhythmias. The relevance of this to safety pharmacology today is that if I_{Na} is a target mediating a proarrhythmic adverse effect of Class I antiarrhythmic drugs, any other drug that blocks ventricular I_{Na} may potentially possess a proarrhythmic liability. Demonstrating this in small animal nonclinical studies with arrhythmia as the primary readout has proven difficult (Farkas & Curtis, 2002).

Harmer et al. (2011) have recently proposed provisional safety margins for QRS prolongation (the ECG outcome of ventricular I_{Na} blockade) based on retrospective assessment of clinical data and a single high throughput in vitro (IonWorks™) approach to assess potency of block of cardiac I_{Na} (encoded by hNav1.5). Although the authors provide a good review of data, adoption of their proposals will require validation studies to establish the quantitative relationship between drug-induced I_{Na} block, prolongation of the QRS interval, and incidence/severity of arrhythmias. This parallels the strictures concerning validation of surrogate readout for evaluating drug-induced TdP liability (see commentary by Gintant, Gallagher, and Pugsley (2011) that discusses this in detail).

2.6. Secondary safety pharmacology

The central nervous, cardiovascular and respiratory systems have been designated as vital, and the tests that address these systems have been designated the “Safety Pharmacology Core Battery” of studies (Pugsley et al., 2008). Core battery study guidance is set out in the ST7A document (U.S. Food & Drug Administration, 2001). This may be supplemented by ancillary studies to evaluate effects on other organ systems such as the genitourinary, renal, gastrointestinal, blood and immune systems (Bass et al., 2004; Pugsley et al., 2008). This, and the choice of test, are driven according to need (i.e., following detection
of effects of potential concern during general preclinical toxicological investigation, or by anticipation based on any special requirements of the target patient population, or by the known adverse effect profile of the drug’s class, or by unexpected findings from clinical studies. The purpose is to provide a greater understanding of effects that may not be fully characterized by limiting studies to those predicated by the S7A guidance on core battery studies. Sjödin, Visser, and Al-Saffar (2011) explore population PK analysis to characterize the feasibility of its application to secondary gastrointestinal (GI) safety pharmacology studies. Population PK is the study of “the sources and correlates of variability in drug concentrations among individuals” — usually the target patient population that receive the drug of interest (Aarons, 1991). Note that in drug development, population PK usually provides for an increased understanding of the quantitative relationships, i.e., concentration–time profiles, which can be used to characterize drug safety and efficacy (Sheiner, 1997). Using paracetamol and sulfapyridine in a double marker method for assessing gastric emptying and small intestine transit time, the authors showed that under fasted and fed conditions, PK of drugs such as atropine and erythromycin in dogs may be modeled using population PK analysis, and that this analysis is more detailed and sensitive compared with standardized non-compartmental analysis. Thus, population PK analysis may provide a suitable non-invasive method for the identification and quantification of drug and food-induced changes in gastric emptying and intestinal transit time with applicability to GI safety evaluation.

3. New methods in development to complement current safety pharmacology studies

A mandate of the SPS and the ICH S7A guidance document (2001) is to encourage “the use of new technologies and methodologies in accordance with sound scientific principles”. Several manuscripts in this issue contain novel methods, applicable to cardiovascular safety studies, which will be briefly discussed. Shiry and Hamlin (2011) review baroreceptor function testing (adapted for use in rat studies from human methods) which permits delineation of a drug’s hemodynamic effects into heart rate versus vascular actions. This is important in view of the potential for an NCE to cause postural hypotension, a highly common clinical adverse event. Cools et al. (2011) characterized the incidence of spontaneous arrhythmias in naive Beagle dogs and the effects of implantation of ECG leads and left-ventricular catheters for telemetry. Long term analysis of ECGs revealed that the prevalence of 2’AVB was 49% and single VPsBs was 28% in drug naive, normal healthy dogs. Implantation of probes into the ventricle for chronic use resulted in an increased frequency of ventricular arrhythmias which resolved to minimal levels – 8 weeks post-surgery. This has important implications concerning study design and the avoidance of false positive readout in proarrhythmia liability testing. Brockway and Hamlin (2011) describe the performance of a novel ECG analysis algorithm (based on Multi-Domain Signal Processing™, MDSP) and show that noise amplitudes can be reduced (up to 85%) preserving ECG signal information content in NHP. The observed standard deviation for the ‘de-noised’ QT interval was reduced by 22% suggesting the potential for increased sensitivity in detection of subtle ECG changes in safety assessment. Green et al. (2011) describe a cellular based approach (ventricular rate adaptation) for assessing drug effects on ventricular refractoriness (another surrogate for TdP). Based on changes to mechanical response (measured optically) drug effects are assessed using rapidly increasing stimulation trains (with pauses, mimicking the short–long–short sequence associated with torsades de pointes initiation) in rabbit myocytes. Proarrhythmic compounds (cisapride, grepafloxxin) prolonged the fastest rate at which the myocyte could no longer respond (i.e., the longest cycle length with incomplete capture) whereas negative control drugs with negligible perceived TdP liability (captopril, loratadine) did not affect rate adaptation. Thus, all the methods described are interesting new approaches to cardiovascular safety assessment.

3.1. In vitro cardiac slice preparations

An interesting in vitro method presented at the SPS meeting described the preparation and use of very thin cardiac tissue slices for TdP liability testing. Slices are proposed to be used to complement better established in vitro cardiovascular models (such as the Langendorff heart, perfused wedge preparation and papillary muscle preparation) (Pugsley et al., 2008). Cardiac tissue slice methods have been improved such that tissue structure and cellular architecture are preserved (Busse et al., 2009). Until recently, the elastic nature of the myocardium made viable thin slice preparation impossible; however, if hearts are perfused with high potassium solution and contractility suppressed, a portion of ventricular tissue can be glued on to an agarose block and slices made with a vibrotome. Vertical transmural slices (~350 μm thick) can be prepared without enzyme digestion permitting up to 72 hour viability when stored correctly (Busse et al., 2009). Action potentials can be recorded, extracellular field potentials measured and excitation determined with multi-electrode arrays in multiple preparations simultaneously using the SYNCHROSICLE (Lohmann Research Equipment, Castrop-Rauxel, Germany). Assay characteristics suggest that electrophysiology parameters in guinea-pig slices are similar to those in papillary muscle preparations (Busse et al., 2009). Pharmacological responses have been evaluated using E-4031, risperidone and nifedipine and effects appear to be qualitatively equivalent to effects reported in myocytes and papillary muscle preparations (Busse et al., 2009). Thus, while promising, the model requires further testing of sensitivity and specificity to standard drugs for validation.

3.2. Blood pressure recording — moving on up… or out?

The ILLUMINATE Phase 3 clinical trial with torcetrapib, in which a slight increase (4 mm Hg) in arterial blood pressure translated into an increase in major cardiovascular events and mortality, highlights the importance of sensitive blood pressure recording in safety pharmacology assessment (Psaty & Lumley, 2008). High definition oscillometry (HDO), the real-time visualization of blood pressure pulse waves measured as oscillations within a cuff bladder (based on the Riva-Rocci papillary principle) has been a considerable item of discussion in safety pharmacology (Pugsley, Towart, Authier, Gallacher, & Curtis, 2010). This method remains under investigation for applicability to non-invasive blood pressure recording in repeat-dose toxicology and safety pharmacology assessment (Egner, 2007; Mitchell, McMahon, Beck, & Sarazan, 2010; Schmelting, Niehoff, Egner, Korte, & Weinbauer, 2009). Although current non-invasive cuff methods of blood pressure recording in conscious dogs and monkeys are difficult, Mitchell et al. (2010) found distinct drug-mediated hemodynamic changes with the HDO method indicating enhanced sensitivity. Hopefully additional follow-up validation studies will be conducted on this novel methodology.

3.3. Utilization of cardiac stem cells in safety pharmacology studies

As discussed previously (Pugsley et al., 2010), model validation remains a vexing issue in safety pharmacology, especially for novel platforms such as stem cells. Models and biomarkers are “valid” only when they detect all and only those drugs that have a safety liability in humans. Thus, no model is truly validated until a range of positive and negative controls have been shown to produce the same outcome in the model as occurs in humans. The in vitro hERG assay using human myocyte cell lines is sensitive (0.82–0.90) in terms of predicting the outcome of a clinical ‘thorough QT’ study, however, specificity is only high at low clinical exposure multiples (i.e., 0.75 at 2× the clinical free plasma concentration) (Wallis, 2010). Moreover, predicting QT
prolongation is not the same as predicting TdP liability (Puglsey et al., 2008), so it may not be wise to attempt to validate a channel screen on the basis of concordance with QT readout, since it is TdP liability that is the real concern.

Alternative hERG screen models to isolated human cardiac myocytes include pluripotent stem cells (Vidarsson, Hyllner, & Sartipy, 2010). The undifferentiated human stem cell of embryonic origin (hESC) and induced pluripotent stem cell (iPSCs) of somatic origin (the latter with the potential advantage that they can be obtained from diseased patients, e.g., congenital LQTS) are being evaluated for cardiac electrophysiological properties and potential for use as a drug screening assay (Peng, Lacerda, Kirsch, Brown, & Bruening-Wright, 2010; Puglsey et al., 2010; Tanaka et al., 2009). Intracellular recordings from individual cells (Peng et al., 2010) and multi-electrode arrays enable measurement of sodium (SCN5A), calcium (Cav1.2) and potassium (\( I_{Kc} \) and \( I_{Kr} \)) currents. The few studies conducted to date suggest that although ion channel profiles in hESC are somewhat similar to those of canine and rabbit Purkinje fibers (Peng et al., 2010), differences include a reduced latency for development of channel block and increased potency for \( I_{Kr} \) block by terfenadine and quinidine (Peng et al., 2010). Application of stem cell technologies to acquired or congenital (usually autosomal dominant) long QT syndromes (LQTS) (of which there are at least 10 variant forms of the syndrome) has been described. This may be useful in that LQTS may predispose to drug-induced TdP (albeit, this is likely to have minimal influence in overall TdP liability in the population). Mutations can occur in the KCNQ1 (or KVLQT1 or Kv7.1) gene and result in a deficiency in \( I_{Kr} \) (slow component of the delayed rectifier current). This phenotype of the LQTS is known as Type I. Recently, Moretti et al. (2010) screened a family affected by the LQT1 phenotype (an R190Q mutation) and used dermal fibroblasts to generate pluripotent stem cells which were subsequently reprogrammed to differentiate into cardiac myocytes. Cell specific markers encoding human transcription factors revealed individual atrial ventricular and nodal cell phenotypes (distinguished by distinct action potentials, AP). “Ventricular” cells derived from patients with LQT1 had APD\(_{90}\) values of 554 ms compared to 373 ms from cells derived from control patients. Electrophysiological characterization of the LQT1-derived cardiac myocytes shows that although they have altered \( I_{Kr} \) activation and deactivation kinetics they show susceptibility to catecholamine-induced tachyarrhythmias suggesting that pluripotent stem cells may be used to study drug-induced TdP liability with KVLQT1-related pathology.

Mutations can also occur in the KCNH2 (or hERG or Kv11.1) gene which results in a deficiency in \( I_{Kr} \) and prolongation of the QT interval (long QT syndrome 2 or LQT2). Itzhaki et al. (2011) and Matsa et al. (2011) have used dermal fibroblasts from patients (with either a G1681A or A614V mutation in the KCNH2 gene, respectively) in order to develop human induced PSC. In both studies the LQT2-iPSC cells exhibited mixed cardiac cell phenotypes with prolonged AP. “Ventricular-like” cell phenotypes showed prolonged APD\(_{90}\) intervals (up to 1300 ms in the Itzhaki et al. study), and the current could be blocked with the potent hERG blocker E-4031 (but was used at concentrations >500 nM). Very oddly, however, the authors used the calcium channel blocker nifedipine (at 1 \( \mu M \)) (and not verapamil or diltiazem in their study) which apparently eliminated the EAD that developed; however, this is a perplexing choice of test drugs since nifedipine predominantly affects vascular smooth muscle. Itzhaki et al. (2011) did show that ranolazine (a ‘late’ sodium current blocker) up to 50 \( \mu M \) did not alter APD\(_{90}\) but eliminated ectopic activity in cells.

Timothy syndrome is a rare multisystem disorder in which patients manifest a prolongation in the cardiac QT interval (in addition to syndactyly and autistic spectrum disorders). The QT effects result from missense mutations in the CACNA1C gene resulting in hyperactivation of the L-type calcium channel (Ca,1.2) (Yazawa et al., 2011), i.e., ion channel inactivation is impaired. Yazawa et al. (2011) used a stem cell ‘recipe’ to differentiate dermal fibroblasts into ‘ventricular-like cardiac myocytes’, as has been done with LQT1 and LQT2. Timothy syndrome ‘ventricular’ myocytes spontaneously beat at rates one half the rate of controls (or only ~30 beats/min), exhibit altered channel inactivation kinetic profiles and have prolonged APD\(_{90}\) values (~3-fold longer than controls) with a propensity to develop delayed after-depolarizations (DAD).

Thus, while these studies are interesting, it is hoped that a comprehensive electrophysiological profile of channels will be conducted along with complete validation studies assessing the ion channel blocking potencies of many standard drugs, at different concentrations, in order to determine IC\(_{50}\) values for comparison with current validated models. Validation is required to ‘establish a case’ for stem cell use in early drug screening or cardiovascular safety pharmacology frontloading. However, in the absence of full data on sensitivity, specificity and predictive values compared with current established non-clinical models the use of stem cells will remain secondary or tertiary in safety assessment.

### 4. Safety pharmacology — development, direction and future of the discipline

Redfern and Valentim (2011—this issue) conducted an analysis of the 1180 posters submitted from inauguration of the Safety Pharmacology Society in 2001 until the recent 2010 meeting. Growth in submissions reflects a 6-fold increase over the decade, with contributions from pharmaceutical companies (~45%) and contract research organizations (~32%) comprising the majority. Cardiovascular-related poster submissions are double that for any other organ system or area of investigation. An interesting analysis of trends has been conducted for in vitro, in vivo and in silico models, methods and approaches applied to safety pharmacology. Also analyses have been conducted for species selection, influence of regulatory guidelines (E14, ICHS6 and ICHS9) along with gap analysis and prediction of future trends in safety pharmacology. Bass et al. (2011—this issue) provide a review that cohesively summarizes the events of the past 10 years that have led to the genesis, evolution of safety pharmacology as a discipline buoyed by a society rich in depth and breadth of expertise reflective of the membership. The article provides sense of the current state of affairs of safety pharmacology but also projects ahead remarking upon future challenges as well as opportunities.

All are encouraged to read these fascinating articles in order to appreciate the development, direction and future of safety pharmacology as a discipline.

### References


