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Appraisal of state-of-the-art

# Methodological innovations expand the safety pharmacology horizon

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ABSTRACT

Almost uniquely in pharmacology, drug safety assessment is driven by the need for elaboration and validation of methods for detecting drug actions. This is the 9th consecutive year that the Journal of Pharmacological and Toxicological Methods (JPTM) has published themed issues arising from the annual meeting of the Safety Pharmacology Society (SPS). The SPS is now past its 10th year as a distinct (from pharmacology to toxicology) discipline that integrates safety pharmacologists from industry with those in academia and the various global regulatory authorities. The themes of the 2011 meeting were (i) the bridging of safety assessment of a new chemical entity (NCE) between all the parties involved, (ii) applied technologies and (iii) translation. This issue of JPTM reflects these themes. The content is informed by the regulatory guidance documents (S7A and S7B) that apply prior to first in human (FIH) studies, which emphasize the importance of seeking model validation.

The manuscripts encompass a broad spectrum of safety pharmacology topics including application of state-of-the-art techniques for study conduct and data processing and evaluation. This includes some exciting novel integrated core battery study designs, refinements in hemodynamic assessment, arrhythmia analysis algorithms, and additionally an overview of safety immunopharmacology, and a brief survey discussing similarities and differences in business models that pharmaceutical companies employ in safety pharmacology, together with SPS recommendations on 'best practice' for the conduct of a non-clinical cardiovascular assessment of a NCE.



## 1. Introduction

This is the first of a two-part issue of JPTM focused on methods in safety pharmacology; especially studies conducted under the auspices of the ICH S7A and S7B guidelines (U.S. Food & Drug Administration, 2001, 2005; Pugsley, Authier and Curtis, 2008; Pugsley, Authier, Towart, Gallacher and Curtis, 2009). This is the 9th consecutive year that JPTM has published a safety pharmacology themed issue. The manuscripts originate from presentations made at the recent Safety Pharmacology Society meeting in Innsbruck, Austria (see Cavero, 2012 for a comprehensive overview of the meeting). The manuscripts provide an update on the broad spectrum of methodological innovation in safety pharmacology around the world. They showcase this innovation and detail important issues concerning the current regulatory environment. They also identify novel areas where safety pharmacology may have larger impact in the future. It is hoped that the articles will help inform an evolving consensus of opinion on best practice in safety pharmacology, and facilitate change where change is required.

## 2. Cardiovascular safety evaluation update

Time required and extent of animal use in research are lessened by including a greater range of readout in a single integrated protocol. Ingram-Ross et al. (2012) provide, for the first time, a comprehensive nonclinical respiratory safety pharmacology assessment using respiratory inductance plethysmography (RIP) and, at the same time, standard cardiovascular (CV) safety assessment in non-human primates (NHP). This 'cardiorespiratory' study provides data that show that the integrated assessment of cardiovascular and respiratory parameters in NHP is achievable continuously for at least 24 h after test article administration. The study design maximizes information obtained from each experiment. This ultimately contributes to a reduction in the total number of animals used, in keeping with the replacement, refinement, and reduction of animals in research agenda (Balls, 1994). Additionally, availability of an integrated protocol may allow safety assessment to be completed earlier in the drug development pathway, facilitating early termination of NCEs with inadequate safety margins.

Guns, Johnson, Weltens and Lissens (2012) conducted an elegant study showing that a negative electro-mechanical window (EMw) determines the risk of NCE-induced *torsades de pointes* (TdP). The EMw defines the relationship between the duration of electrical

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systole (which was measured indirectly as QT interval) and that of mechanical systole (which is measured indirectly as the time between the Q wave and the second heart sound; QS2) (De Caprio et al., 1984; Fabritz, 2007; van der Linde et al., 2010). Altered autonomic tone or circulating catecholamine levels is an associated indicator of cardiovascular disease and Van der Linde et al. (2010) showed in their fentanyl-etomidate anesthetized beagle (FEAB) dog model that it is not the prolongation of the QTc interval but rather the development of a large negative EMw that could provide the necessary condition(s) that subsequently manifest as TdP (albeit in an adrenergic-dependent manner). The anesthetized guinea pig model as described by Guns and colleagues could be used in early frontloading studies as it has many advantages including a requirement for minimal amounts of test article and reduced cost. The work nicely complements previously published findings from anesthetized canine studies conducted by van der Linde et al. (2010).

Ward, Milliken, Patel and McMahon (2012) compared non-invasive (Jacketed External Telemetry or JET™) and implanted telemetric measurement of blood pressure and the ECG in conscious beagle dogs. Findings with test articles used [$N^G$-nitro-L-arginine methyl ester (L-NAME) and minoxidil] suggest that the non-invasive JET™ system can be used to acquire blood pressure and ECG data simultaneously for extended periods of time (~26 h); however, the JET™ method still requires refinement in order to improve sensitivity to detect small changes in blood pressure. The JET™ system is limited because it requires the use of the oscillometric tail cuff method to determine changes in blood pressure. Implications of such work include the potential for 'real-time', fast analysis of blood pressure in concert with the ECG and potentially further integration of core battery methods — to include a hemodynamic component.

The choice of animal species and cardiac tissue type to be used in safety pharmacology studies is made difficult in part by the range of options available. A comprehensive understanding of the literature and complexity of the profile of ion channels within the various anatomical regions of the heart from the different animal species is a mandatory requirement in order to fully exploit the output generated from such studies. Without prior experience in the use of tissues from multiple species, grievous errors in drug risk assessment could arise. Lu, Vlaminckx, Cools and Gallacher (2012) provide an excellent example of such an evaluation by examining the direct/acute effects of arsenic trioxide ($As_2O_3$), used clinically in acute promyelocytic leukemia (Ficker et al. 2004). Arsenic trioxide is a notoriously difficult compound to evaluate in non-clinical cardiac models because of its complex time–exposure relationship and variable concentration-dependent properties for ion channel blockade and modulation of action potentials (AP). Lu et al. (2012) characterize its effects in isolated cardiac tissues from four different species (guinea pig, rabbit, dog and porcine). Study results indicate that the choice of animal species, cardiac tissue and study conditions can affect outcome and are critical to inform 'go/no-go' decision making in safety assessment.

Recently the SPS was introduced to the use of cardiac tissue slices (Bussek et al., 2009; Pugsley et al., 2011) as an alternative *in vitro* method with which to study the effects of a NCE on action potential parameters involved in cardiac depolarization and repolarization. Cardiac slices are a novel methodology proposed to complement better established *in vitro* cardiovascular models (e.g., Langendorff heart or perfused wedge preparation) (Pugsley et al., 2008). Since this development, cardiac tissue slice methods have been refined to preserve tissue structure and cellular architecture (Bussek et al., 2009) and even a human variant exists (see Curtis, 2012). Bussek et al. (2012) reveal how this screening method (using rat and guinea pig hearts) may be robust enough to be used to assess the effects of long duration (i.e., up to 28 h) tissue exposure to the NCE. Cardiac slices as well, may be used for several days after preparation if maintained with appropriate buffers and storage conditions. Further validation is required but this is an interesting model to possibly complement current methods for early cardiovascular safety assessment (see Pugsley, Towart, Authier, Gallacher and Curtis, 2010 for details).

## 3. Cardiac contractility — a new cardiovascular safety pharmacology study endpoint?

With heart rhythm and blood pressure dominating consideration in cardiovascular safety assessment, cardiac (ventricular) contractility has been neglected and has only recently become of potential interest in the cardiovascular safety milieu (Sarazan et al., 2011). Contractility is largely omitted from pre-clinical regulatory submission packages by most pharmaceutical companies. Recently however it has become a topic of conversation at SPS meetings, and a strategy for its consideration is under discussion. Contractility would initially represent a readout complementary to the current cardiovascular endpoints assessed under the core-battery ICH S7A guidance, but this could change. Several papers have recently been published that evaluate direct measures of contractility (*in vivo* — versus that recorded from isolated Langendorff hearts). Contractility variables include left ventricular pressure (LVP) and rate of contraction and relaxation ($\pm dP/dt_{max}$) recorded directly via invasive catheter implantation and/or non-invasive echocardiography. Unfortunately, the former method can cause arrhythmias and the latter may be cost prohibitive. Therefore interest has focused on an indirect measure of contractility derived from the ECG and the blood pressure line: the QA interval. The QA interval is the time interval between the onset of the Q-wave and the onset of the upstroke of the aortic pulse pressure wave. Hamlin & del Rio (2012) provide an overview of myocardial contractility and the fact that dP/dtmax may sometimes be an inappropriate measure of the inotropic state of the myocardium, unless adjusted for pre-load (LV end-diastolic volume). The authors coin the term *baroinometry* in order to reflect the fact that dP/dtmax pertains to aortic pressure (*baro*), the inotropic state (*ino*), and the length (*meter*) of the myocardium. In separate studies cognizant of the force–frequency relationship of inotropy and its potential confounding influence on contractility safety assessment, Markert et al. (2012) have determined species-specific mathematical formulae in order to correct myocardial contractility (LV $dP/dt_{max}$) for changes in heart rate in conscious dogs, primates and minipigs. The relationship between LVdP/$dt_{max}$ and HR was evaluated for each species to generate formulae for drug-free regression, and drug validation was undertaken with a negative (itraconazole) and positive (pimobendan) inotrope. The authors found that it was possible to assess the inotropic effects of drugs independently of concurrent variations in HR.

Sarazan, Kroehle and Main (2012) discuss basic principles of cardiac contractility (such as inotropism, $dP/dt_{max}$, and measurement of LVP) and some of the basic principles of physics and calculus used to record contractility signals from dogs, non-human primates and rats. This article reviews some very complex aspects of the components (such as sample rates and noise) inherent to the data acquisition systems used in the recording of cardiac contractility signals. The review provides interpretation guidance to very complex topics in a highly palatable manner for typical readers of JPTM.

## 4. Large pharmaceutical company operation survey and SPS best practice recommendations

Results from an anonymous survey was sent out to safety pharmacology representatives at the largest 12 pharmaceutical companies (as defined by annual revenue) are described in an article by Ewart et al. (2012). The survey sought to understand how safety pharmacology operates within the larger companies and how the operational aspects (e.g., such as group size, accountabilities, roles and responsibilities of group members, outsourcing policy and publication record) of this relatively young discipline compare. Many similarities were found, but safety pharmacology group size was glaring differently

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between companies. A majority of companies (~54%) employs 10–30 full time equivalents (FTE's) in safety pharmacology, but group size ranged from less than 10 to more than 50 FTEs.

Leishman et al. (2012), on behalf of the SPS, assembled over 50 experts to discuss best practices in the conduct and reporting of key nonclinical cardiovascular safety pharmacology (and toxicology) assessments in drug development. Recommendations were provided concerning provision of details of the models and analysis methods used, the model sensitivity and the power of study designs, consideration of how well readout fits with historical data, and more specific issues such as background arrhythmia incidence associated with the use of the species studied (e.g., dogs vs. primates). This is a highly informative article that provides the regulator, clinician, academic scientist as well as the student with a blue-print for evaluating whether a cardiovascular safety study is fit for purpose.

## 5. Ventricular arrhythmia review and analysis

The analysis and quantification of cardiac arrhythmias are complex (Curtis & Walker, 1988). Arrhythmias in non-clinical studies are usually categorized according to the Lambeth Conventions (Walker et al., 1988) which provides guidance on design and analysis of studies. It also provides definitions of major arrhythmias including ventricular premature beats (VPB) bigeminy, salvo, ventricular tachycardia (VT) and ventricular fibrillation (VF). However, the Lambeth Conventions does not provide specific guidance on the complexity of arrhythmia assessment in safety pharmacology.

Cools et al. (2011) previously characterized the incidence of spontaneous arrhythmias in naïve beagle dogs and the effects of implantation of ECG and left-ventricular catheters for telemetry. Long term analysis of ECG's identified the prevalence of 2nd degree AVB (49% incidence) and single VPBs (28%) in studied dogs. Implantation of probes into the ventricle for chronic use resulted in an increased frequency of ventricular arrhythmias. A recent equivalent article by Chui, Derakhchan and Vargas (this issue) provides a comprehensive analysis of cardiac arrhythmias in telemetered non-naïve (drug-free) Cynomolgus monkeys evaluated over a 6 month period. Arrhythmias were analyzed using pattern recognition software (ecgAUTO™) that identifies changes in rate and rhythm, using the PR interval as an indicator of aberrant rhythms and identifying VPBs from a master waveform library. In terms of data, it is not surprising that monkeys exhibited a variety of low frequency, spontaneous arrhythmias which appeared to vary diurnally. VPB occurrence was observed in 25% of animals during the day but in only 12% of animals at night. More severe arrhythmia incidence (e.g., VT) was observed in only 1.4% of animals during the day. Thus, researchers are advised that in order to limit the potential for such arrhythmias to confound NCE assessment, prescreening ECGs must be scrutinized given that arrhythmias may be inherently present in animals prior to and especially after the surgical implantation of telemetry catheters into animals. In our view this issue may be a red herring to a certain extent since a false positive can only occur if the study is done as a 'before vs after' assessment in a single group of animals, or if the study is underpowered. A randomized blinded study between groups of adequate size cannot falsely identify proarrhythmia even if there are spontaneous arrhythmias present prior to dosing.

Koeppel, LaBarre and Zitoun (2012) have developed a new software application named ARR30a, a module from the NOTOCORD-hem data analysis software platform that provides a reliable, fast, automated detection system that can be used in preclinical studies to detect ventricular premature beats (VPBs). The software was validated using an ECG database constructed with numerous ECG recordings representative of preclinical data. The selected species included non-human primates, pigs/minipigs, dogs, guinea pigs and rats. Five major arrhythmia types were targeted for detection: sinus pause, atrial beats, junctional beats, VPBs and 2nd degree atrio-ventricular block (AVB). Arrhythmia detection sensitivity and positive predictivity were evaluated by comparing with manual analysis. In dogs, non-human primates and pigs, ARR30a sensitivity was 91%, 82% and 78% for determining VPBs, AVB and junctional beats with an associated predictivity of 83%, 94% and 94%, respectively. With further validation ARR30a may be useful in the evaluation of arrhythmias associated with assessment of a NCE.

## 6. Safety immunopharmacology and beyond

Safety immunopharmacology is a new topic for JPTM. Descortes (2012) provides an overview of the ICH S6R1 and S8 guidance documents for the evaluation of a biological agent and NCE, respectively. Immune-mediated adverse effects are discussed in terms of immunosupression, immunostimulation, hypersensitivity and autoimmunity. In addition, a review of short-term (≤7 days) pharmacology studies that can be used to evaluate the immunological safety profile of a pharmaceutical is presented. It is hoped and expected that methods will be developed and validated for all aspects of safety assessment in the coming years. These methods will be reported in JPTM in future volumes.

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