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Original article

Reprint of "Safety pharmacology in 2014: New focus on non-cardiac methods and models" ☆

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ABSTRACT

"What do you know about Safety Pharmacology?" This is the question that was asked in 2000 with the inception of the Safety Pharmacology Society (SPS). There is now a widespread awareness of the role of safety pharmacology in drug discovery and increasing awareness among the wider community of methods and models used in the assessment of the core battery required set of safety studies. However, safety pharmacology does not stop with core battery studies. New methods are intensively sought in order to achieve a swifter and more reliable assessment of adverse effect liability. The dynamics of the discipline and method expansion are reflected in the content of this issue of the *Journal of Pharmacological and Toxicological Methods* (JPTM). We are into the second decade of publishing on safety pharmacology methods and models, reflected by the annual themed issue in JPTM, and on willingness of investigators to embrace new technologies and methodologies. This years' themed issue is derived from the annual Safety Pharmacology Society (SPS) meeting, held in Rotterdam, The Netherlands, in 2013.

1. Overview

As with the previous JPTM SPS themed issues, papers consider some exciting new applications with attempts to validate *in vitro* and *in vivo* safety pharmacology models. Surprisingly (since historically safety pharmacology method innovation has been focused on cardiac adverse drug reactions (ADRs)), the majority of the articles in this issue probe CNS drug safety methods. Topics range from the introduction of behavioral test methods into sub-chronic (≤3 month) regulatory toxicology studies to the characterization of the response of non-human primate sleep architecture and EEG activity to known pharmacological agents (caffeine, amphetamine and diazepam) using telemetry-based polysomnography. Likewise, the use of telemetry video electroencephalography (EEG) in rats, dogs and non-human primates is discussed as a method in 'follow-up' safety pharmacology seizure liability assessments for new chemical entities (NCE). Finally, the utilization of both *in vitro* (hippocampal slices) and *in vivo* (rats implanted with EEG electrodes for monitoring via telemetry) methods were complementary in characterizing the seizure potential of an NCE. Respiratory articles include a review that discusses the value and utility of the methods and measurement endpoints currently available for assessing respiratory function to help optimize the design of respiratory safety pharmacology studies and readers are introduced to airwave oscillometry, which appears to be a promising non-invasive methodology to measure respiratory mechanics in conscious animals. In the day and age of stem cells and their possible future application to drug safety assessment we include an article that investigates the actions of E-4031, verapamil, dofetilide, pentamidine, terfenadine, quinidine and nifedipine on action potentials (AP) and ion currents recorded from adult human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). hiPSC-CM are shown to display excellent sensitivity to ion channel blockers resulting in characterized changes in the electrophysiology of the AP suggesting use as a screening method for NCEs. Another article predicts the result of the human clinical Thorough QT (TQT) study using *in silico* methods applied to data derived from multiple ion channel screens. *In silico* AP simulations were conducted using many models and findings suggest that this approach is a useful complementary tool in cardiac safety assessment. Lastly, also included in this issue is a comprehensive review on cardiovascular pressure measurement and its application to safety assessment studies. The article nicely highlights the technology requirements and discusses the potential errors that can be made from such recordings.

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2. Methods used to assess CNS function in the safety pharmacology assessment of a new chemical entity (NCE)

When compared to the cardiovascular system the other required vital core battery components, the CNS and respiratory organ systems, albeit important, are much less intensely explored in the context of methodological innovation. The predominance of the cardiac system in forums and scientific literature on safety pharmacology is at least partially attributable to the high impact of rare but lethal events related to this system (Curtis & Pugsley, 2012; Valentin & Hammond, 2008). Interestingly, therefore, this issue contains a variety of articles that highlight innovative methods used to assess CNS, respiratory and systems relevant to safety assessment of an NCE.

As per ICHS7A (US FDA, 2001), non-clinical safety pharmacology studies generally evaluate a single dose of test article or doses ranging from efficacy to low multiples above efficacy, which impinge upon but do not result in overt end organ toxicity (as this can limit interpretation of the drug-induced pharmacological effects on cardiovascular (CV), respiratory and CNS function). Safety pharmacology studies are conducted in accordance with good laboratory practice (GLP) and use the most relevant state of the art methods to assess CV changes, respiratory function or CNS liability while ensuring that optimal study conditions are considered (Guth et al., 2009; Pugsley, Authier, & Curtis, 2008). The assessment of CV, respiratory and CNS effects of an NCE in toxicology studies, however, is limited. Because toxicology studies (acute, subchronic and chronic) are critical in assessing the end organ toxicological response of an NCE, a conservative stance has existed with respect to modification of study design and application of new technologies and methodologies (see Authier, Vargas, Curtis, Holbrook, & Pugsley, 2013 for SPS survey of best practices on this topic). Toxicology studies do not usually involve the use of animals instrumented for telemetry monitoring of CV function because CV assessment is ancillary when the focus is on toxicity. However, Golozoubova et al. (2014) incorporated a safety pharmacology behavioral (i.e., modified Irwin screen) test into a standard sub-chronic (3 month) toxicology study with a view to reduction of animal use (3 Rs principles) by replacing stand-alone safety pharmacology experiments in the assessment of an NCE. The authors used two strains of rat (Wistar and Sprague-Dawley), of both genders, and found little differences in response; however, they observed that with time, individual animal variability actually increased with repeated measurements. The authors suggest that researchers conducting such studies need to consider study design and animal group size if they are to integrate safety pharmacology endpoints into toxicology studies (see article by Pugsley, Towart, Authier, Gallacher, & Curtis, 2010). This resonates with other emerging wider pharmacological methodological guidance (Curtis et al., 2013).

Authier et al. (2014a, 2014b) applied the clinical diagnostic method of polysomnography, i.e., the comprehensive recording of brain (electroencephalogram, EEG), eye movements (electro-oculogram, EOG) and muscle activity (electromyogram, EMG) during sleep to nonhuman primates (NHP). Use of such methods is hoped to enhance the translational potential of drug-induced changes in sleep architecture in non-clinical species to patients. The authors characterized the responses of known pharmacological agents (d-amphetamine, diazepam and caffeine) that affect sleep structure and EEG activity in NHP (*Macaca fascicularis*) using telemetry-based polysomnography. Animals in the study were instrumented with telemetry transmitters for continuous recording of EEG, EOG and EMG monitoring (combined with video). At the doses tested all observations were similar to those previously reported for the pharmacological effects observed in humans suggesting that telemetry monitoring of EEG, EOG and EMG in the NHP could be a useful non-clinical approach with which to investigate drugs with the potential to induce sleep disturbance.

Bassett, Troncy, Pouliot, et al. (2014a) examined non-clinical seizure liability using intravenous pentylenetetrazole (PTZ) in combination with pharmacological agents that alter seizure thresholds and induce clonic convulsions in EEG telemetered Cynomolgus monkeys, Beagle dogs and Sprague-Dawley rats. The authors outline and evaluate the premonitory clinical signs in each species that include altered physical activity, enhanced tremors, ataxia, emesis and myoclonus. Drugs tested included amphetamine, caffeine, yohimbine and phenobarbital and the results provided a clear template for identifying pro- and anti-convulsant activity that may be useful in CNS safety assessment of an NCE. Importantly, the authors emphasize that when seizure liability investigations are conducted, rats typically represent the 'first-line' model whereas Beagle dogs are generally overly sensitive to seizure susceptibility (Edmonds et al., 1979) (and are typically only used if required by regulatory authorities).

Markgraf et al. (2014) described the use of *in vitro* and *in vivo* non-clinical models in the evaluation of the seizure potential of a CNS-targeted NCE. They used Org 306039, a potent and selective 5-HT2c agonist that was in development for obesity as a positive control in both types of assays. The 5-HT2c receptor is associated with excitatory neurotransmission (i.e., the binding of serotonin to the receptor subtype inhibits dopamine and norepinephrine release) in the prefrontal cortex, hippocampus, hypothalamus and striatal brain regions. These receptors are reported to modulate mood and anxiety and have been in development as an ensemble target for anti-obesity and antidepression. The authors used rat hippocampal slice preparations and male Sprague-Dawley rats implanted with telemetry EEG recording electrodes to characterize responses to the seizure causing standard, Org 306039. Animals received either vehicle or Org 306039 (100 mg/kg, po) daily for 10 days. Org 306039 elicited a concentration-dependent increase in population spike area and number recorded from hippocampal CA1 cells, indicative of an increase in potential seizurogenic activity. Repeat dosing of Org 306039 was associated with the appearance of 'seizure-related' behaviors (such as shivering, tremors and head shakes) and pre-seizure waveforms on the EEG with the observation of an overt seizure in one animal. Findings from both the hippocampal slice preparations and *in vivo* models appear complementary to one another when used in the characterization of the seizure potential of CNS-targeted compounds.

3. Recommendations and novel methods used to assess respiratory function

Core battery safety pharmacology respiratory studies focus on drug effects on basic pulmonary function (Pugsley et al., 2008). The standard model utilizes whole body plethysmography recording in conscious rodents (Murphy, 2005). In this issue of the *Journal*, Murphy (2014) describes, in a comprehensive review article, the variety of methods and pulmonary functional endpoints that can be used in the evaluation of an NCE for effects on respiratory function. In order to conform to the ICH S7A guidelines, respiratory safety pharmacology studies generally are conducted using conscious animal models and usually assess pulmonary ventilation including respiratory rate (RR), tidal volume (Vt) and arterial blood gases — standard variables. These measures describe pulmonary ventilation (or minute volume, MV), frequency of breathing (RR), and depth of breathing (Vt) and are surrogates for adequate gas exchange and tissue oxygenation (Murphy, 2013). However, as Murphy discusses, other variables that can be used to provide mechanistic insight or identify site of drug action, should also be considered for incorporation. These variables include inspiratory and expiratory times, flows (and pauses) as well as apneic time. However, measures of pulmonary ventilation only assess drug effects on the respiratory pumping apparatus and do not assess other components of the respiratory system such as exchange. These are best characterized by measuring drug effects on airway patency, gas diffusion capacity and lung elastic recoil. These additional measures are not commonly determined in standard safety pharmacology respiratory studies. It is useful to consult the Lindgren et al. (2008) survey that benchmarked safety pharmacology best practice for use in regulatory package submissions. When

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