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Invited Review Article

Safety pharmacology — Current and emerging concepts

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ABSTRACT

Safety pharmacology (SP) is an essential part of the drug development process that aims to identify and predict adverse effects prior to clinical trials. SP studies are described in the International Conference on Harmonisation (ICH) S7A and S7B guidelines. The core battery and supplemental SP studies evaluate effects of a new chemical entity (NCE) at both anticipated therapeutic and supra-therapeutic exposures on major organ systems, including cardiovascular, central nervous, respiratory, renal and gastrointestinal. This review outlines the current practices and emerging concepts in SP studies including frontloading, parallel assessment of core battery studies, use of non-standard species, biomarkers, and combining toxicology and SP assessments. Integration of the newer approaches to routine SP studies may significantly enhance the scope of SP by refining and providing mechanistic insight to potential adverse effects associated with test compounds.

Abbreviations: ADR, Adverse Drug Reaction; ALP, alkaline phosphatase; AKI, acute kidney injury; ALT, alanine aminotransferase; AP, action potential; AST, aspartate aminotransferase; BP, blood pressure; BUN, blood urea nitrogen; CLU, clusterin; CNS, Central Nervous System; CVS, Cardiovascular System; ECG, Electrocardiogram; EEG, electroencephalography; EMA, European Medicines Agency; FDA, Food and Drug Administration; FOB, Functional Observation Battery; GFR, Glomerular Filtration Rate; GGT, γ-glutamyl transferase; GI, Gastrointestinal; GST, glutathione S transferase; hERG, human Ether-a-go-go related gene; hESC, human embryonic stem cells; HR, heart rate; ICH, International Conference on Harmonisation; KIM-1, kidney injury molecule-1; LDH, lactate dehydrogenase; miR, microRNA; β-NAG, *N*-acetyl-β-*D*-glucosaminidase; NCE, New Chemical Entity; NGAL, Neutrophil gelatinase-associated lipocalin; NMR, Nuclear Magnetic Resonance; PBPK, physiologically based pharmacokinetics; PEB, photoelectric beam interruption technique; RPA-1, renal papillary antigen-1; SP, Safety Pharmacology; TFF3, trefoil factor 3; VQM, Ventilation (V)/perfusion (Q) mismatch (M).

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Contents

Introduction

Non-clinical pharmacological studies, including primary pharmacology, secondary pharmacology and safety pharmacology (SP), are an essential element of the drug discovery and development process. Unlike primary and secondary pharmacology studies that explore the mode of action of the candidate drug and its effects related or unrelated to the therapeutic target, respectively, SP identifies the "potential undesirable pharmacodynamic effects of a substance on physiological functions in relation to exposure in the therapeutic range and above" (FDA, 2001) which are not identified by standard non-clinical toxicological studies. SP studies are, therefore, performed to ensure the safety of clinical participants in first in human (FiH) trials (Pugsley et al., 2008) through improved decision-making in the selection of lead candidate drugs. Efforts to standardize SP studies resulted in multiple guidelines from the International Conference on Harmonisation (ICH) including ICH S7A and S7B (FDA, 2001, 2005). The core battery SP studies, performed according to good laboratory practice (GLP) standards as per the ICH guidelines, involves the investigation of the major vital organ systems including the cardiovascular system (CVS), central nervous system (CNS) and respiratory system. In addition, supplemental studies investigating the renal and gastrointestinal (GI) systems and other organ specific follow-up investigations may compliment the core battery studies. However, these are optional and their conduct is determined by the nature of the lead candidate drugs being tested and the type of adverse events anticipated.

SP studies were generally performed during the drug development stage on the selected candidate drug prior to FiH trials. Currently, the onset of SP studies has shifted towards the early drug discovery process (Fig. 1). Thus, SP studies in addition to assessing and mitigating risks associated with the selected candidate drug can now facilitate lead candidate selection by hazard identification and elimination of new chemical entities (NCE) with safety liabilities (Valentin et al., 2009). The purpose of this review is to provide a combined and comprehensive overview of both current practices and newer technologies, followed by the emerging concepts in SP studies: frontloading, alternate models, integrated core battery assessments, integration of SP endpoints into regulatory toxicology studies, drug–drug interactions and translational SP.

Core battery organ systems and studies

Cardiovascular system

In the last few decades, a large number of drugs have been withdrawn from the market due to adverse cardiovascular system (CVS) effects, which were responsible for 45% of post-approval withdrawals (Laverty et al., 2011). The electrical activity in the CVS can be measured using electrocardiogram (ECG), which is analysed by dividing the recorded trace into waves and intervals with particular focus on the QT interval which represents cardiac repolarisation. It is important to note that QT prolongation has resulted in one third of all

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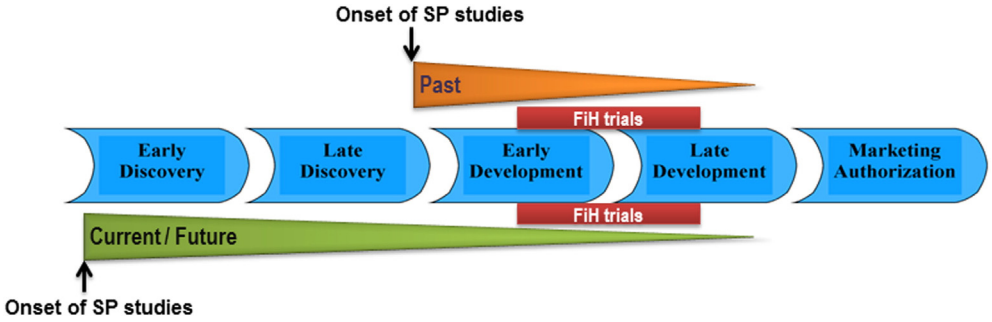

Fig. 1. Safety pharmacology study approaches. Initially, SP studies were conducted after lead candidate identification to profile safety risks in humans according to GLP compliance. In addition, more recent strategy is to initiate SP studies (non-GLP) much earlier in the drug discovery process aims to identify hazardous NCEs facilitating lead candidate selection. This ensures the reduction of risks in humans and lead candidate attrition. FiH — first in human, GLP — good laboratory practice.

drug withdrawals between 1990 and 2006 (Shah, 2006) due to the risk of developing fatal arrhythmias. An example of a drug that caused numerous fatalities due to QT prolongation is terfenadine (Monahan et al., 1990), this led to the implementation of the ICH S7B guidance that describes a "non-clinical testing strategy for assessing the potential of a test substance to delay ventricular repolarisation" (FDA, 2005). Consequently, a core battery of SP tests, consisting of an in vitro assay to assess the extent of the human Ether-a-go-go Related Gene (hERG) potassium channel, $K_v11.1$, blockade, in vivo telemetry and additional in vitro/ex vivo tests were adopted to evaluate the likelihood of an NCE to cause adverse CVS effects (Table 1).

In vitro hERG assay

There is considerable focus on the promiscuous hERG channel, which mediates an inward current, that, when blocked, slows myocardial repolarisation associated with prolongation of the QT interval in the ECG. This prolongation lengthens the duration of the cardiac action potential (AP) (Curran et al., 1995), which appears to be a critical contributing factor in the development of a fatal arrthymia: Torsades de Pointes (Redfern et al., 2003). The effects of an NCE on the hERG channel can be detected using screening methodologies such as radio-labelled ligand binding and automated voltage clamp assays. Alternatively, the manual in vitro electrophysiology patch clamp assay is used to quantify NCE-induced hERG inhibition with a strong accuracy rate for predicting in vivo CVS toxicity (Hancox et al., 2008). However, this in vitro assay is not without limitations, since the hERG channel may be functionally compromised through related, poorly understood molecular mechanisms (Kaczorowski et al., 2011).

In vivo telemetry

In general, physiological data obtained from conscious, large mammals (e.g. dogs, minipigs and non-human primates) is accepted as the gold standard for detecting any effects of an NCE on CVS functionality. Telemetry is efficiently utilised in SP to produce reliable data sets while using as few animals as possible (Samson et al., 2011). Furthermore, it allows the measurement of CVS parameters in conscious freely moving animals with minimal stress. Telemetry can be divided into two distinct techniques: 1) *Jacketed* (*or External*), a non-invasive technique which records ECG parameters and 2) *Implanted* (*or Internal*), an invasive technique requiring surgery, which can simultaneously measure ECG, haemodynamic parameters, such as blood pressure (BP) and contractility, and body temperature. Additionally, telemetry can be used for the simultaneous measurement of other core organ system parameters.

Telemetric devices are used for the continuous measurement of arterial, systemic and left ventricular BP, heart rate (HR) and ECG parameters: the QRS complex and the QT, ST and PR intervals. Since the PR and QT intervals are influenced by the HR, they should be corrected using the relevant formula, determined by the study design and species used. In general, van de Water's correction is used for dogs and minipigs, while Fridericia's or Bazett's corrections are used in either non-human primates or guinea-pigs, depending upon the experimental conditions. However, due to significant inter-individual variation (Malik et al., 2002), an individual correction formula that utilises a complex model of linear regression is applied; however, it requires a large number of HR measurements to obtain an acceptable level of accuracy (Couderc et al., 2005). Finally, other factors such as changes in body temperature and plasma concentrations of electrolytes (e.g. potassium), glucose and insulin, should be taken into account when interpreting ECG readouts.

In vitro isolated myocardial systems

The effects of NCEs on the cardiac AP can also be investigated using other in vitro systems including isolated myocardial tissue (purkinje fibres or papillary muscles) or whole isolated hearts. For example, a functional in vitro model using isolated guinea-pig papillary muscles can be used to evaluate direct NCE-induced effects, including the force of contraction and refractory period, in addition to effects on the AP (Kagstrom et al., 2007). However, these low-throughput techniques are costly and require highly skilled electrophysiologists.

Table 1
Tests and parameters available to assess CVS safety pharmacology. The table outlines the core and follow-up CVS associated parameters in SP testing. It also lists out the established and emerging techniques associated with these investigations. hERG — human ether-à-go-go-related gene; IC_{50} — half maximal inhibitory concentration; HR — heart rate; BP — blood pressure.

Cardiovascular system (CVS) assessment	
Readouts	
Core	Follow-up
In vitro hERG assay (hERG IC_{50})	In vitro isolated organ preparation
Telemetry (HR, BP)	
Established techniques	
In vitro	
hERG assay	
Manual patch clamp	
Automated high-throughput patch clamp	
Isolated organ preparation	
Whole heart preparation	
Isolated purkinje fibres	
In vivo	
Telemetry	
Internal (surgical implant)	
External (jacketed)	
Emerging techniques	
In vitro	
Assays for other ion channels	
Automated high-throughput patch clamp	
Human embryonic stem cell derived cardiomyocytes	
Human induced pluripotent stem cell derived cardiomyocytes	
Telemetry	
Internal	
Femoral artery cannula	
External	
High definition oscillometry	

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Newer technology

Technological advancements have led to the improvement of automated patch clamp assays and this has been beneficial for in vitro CVS studies by facilitating lead candidate optimisation during the drug discovery and development process. There are now a number of commercially available high-throughput automated patch clamp platforms that utilise planar array technology, which can rapidly quantify the degree of an NCE's hERG blockade (Dunlop et al., 2008). While the benefit of being able to screen large numbers of NCEs rapidly is alluring, it is difficult to obtain accurate test concentrations during the screening process. Therefore, this platform should be used in conjunction with other methodologies (Guth and Rast, 2010).

In addition to hERG, the cardiac AP is also regulated by the activity of other ion channels, many of which may also be part of a vulnerable cellular pathway. Some of the following channels have been implicated in other cardiac arrthymias: the slow delayed rectifier potassium channel ($hK_v7.1$/hKCNQ1/hminK); voltage gated potassium channel ($hK_v1.5$); voltage gated sodium-permeable channel ($hNa_v1.5$); hyperpolarisation-activated cyclic nucleotide-gated channel (hHCN4); potassium-permeable outward voltage gated potassium channel ($hK_v4.3$/hKChIP2); L-Type calcium channel ($hCa_v1.2$) and inwardly rectifying potassium channel ($hK_{ir}2.1$) (Grant, 2009; Nattel and Carlsson, 2006). Electrophysiological investigations of these ion channel subunits can also be conducted using the above mentioned electrophysiological techniques (Laverty et al., 2011). This data can provide more informative SP profiles for NCEs for lead candidate development.

Previously, implanted telemetry was required to record CVS parameters, but recently, jacketed ECG telemetry in combination with novel high definition oscillometry methodologies for BP recordings is used as an alternative. Although high definition oscillometry is non-invasive and cheaper than implanted telemetry (Meyer et al., 2010), there are short-comings that include: 1) lower signal to noise ratio; 2) shorter duration of recordings; and 3) lack of in-depth pharmacological validation. However, there are now BP measurement techniques that only require a small transducer to be inserted into the femoral artery (McMahon et al., 2010). Finally, it is important to monitor circadian rhythms, particularly in rodents as blood pressure peaks during the night when activity is highest (Lemmer et al., 1993).

Central nervous system

Adverse drug reactions (ADRs) associated with the central nervous system (CNS) represent a major cause for concern for pharmaceutical companies. A variety of clinically used drugs such as anti-histamines (e.g. diphenhydramine) and benzodiazepines (e.g. diazepam) exhibit common CNS side effects including sedation, ataxia and nausea (Porsolt et al., 2006). More importantly, however, 10% of all drugs withdrawn from the market between1960 and 1999 were due to severe CNS adverse effects (Fung et al., 2001). Therefore, it is beneficial for the pharmaceutical industry to detect these ADRs early in the drug discovery and development process in order to save time and reduce costs, ultimately leading to the design of clinically safer compounds (Pugsley et al., 2008). For this reason, the CNS has been included in the regulatory guideline ICH S7A (FDA, 2001). The effects of NCEs on the CNS are evaluated using a variety of core battery SP studies as outlined by the ICH to detect potential undesirable pharmacodynamic effects on various neurophysiological functions such as "motor activity, behavioural changes, coordination, sensory/motor reflex responses and body temperature" (FDA, 2001). Unlike CVS SP assessments, CNS core battery studies are generally performed using unanaesthetised animals, primarily rodent models (Porsolt et al., 2006). The various established and emerging techniques used to assess neurological functions in CNS SP are depicted in Table 2.

Table 2
Tests and parameters available to assess CNS safety pharmacology. Table outlines the core and follow-up CNS associated parameters in SP testing. It also lists out the established and emerging techniques associated with these investigations.

Central nervous system (CNS) assessment	
Readouts	
Core	Follow-up
Behaviour	Higher cognitive function
Locomotor activity	Seizure liability
Motor co-ordination	Drug abuse
Sensorimotor reflexes: nociception	Drug dependence
Established techniques	
Modified Irwin's test, Functional Observation Battery (FOB)	
Photoelectric beam interruption systems	
Rotarod	
Hot plate test, Tail flick, paw pressure	
Morris maze and passive avoidance tests	
Electrocerebral silence threshold and pentylenetetrazol seizure tests	
Electroencephalography (EEG)	
Self administration and drug discrimination lever chamber models	
Drug withdrawal: FOB, body temperature, body weight	
Emerging techniques	
Automated video systems	
Integrated video and EEG systems	
In vitro hippocampal brain slice assay	
Telemetry	

Behaviour

Procedures for assessing the effect of NCEs on behaviour and physiological state were first described by Irwin in the late 1960s (Irwin, 1968). The Irwin test consists of the systematic evaluation of a battery of general behavioural and physiological observations in the rodent including arousal, vocalisation and stereotypy. Drug treated animal groups are compared to a vehicle group and observational differences between the groups are documented using a qualitative scoring system (Porsolt et al., 2006). Although this methodology provides satisfactory assessment of gross behavioural changes it does not encapsulate other vital neuro-physiological functional assessments outlined by the ICH. As a result the Irwin test has been differentially modified by various drug companies to incorporate all core battery functions detailed in the ICH guidelines (Porsolt et al., 2006). Similarly to the modified Irwin's test, the Functional Observation Battery (FOB) provides a more comprehensive evaluation of NCEs on the fundamental CNS functions (Table 3). Additionally, FOBs are frequently used to carry out neurotoxicological and neuropathological investigations (Shell et al., 1992). Drugs, such as the psychostimulant, amphetamine, and the antipsychotic, chlorpromazine, can be used as reference compounds to validate the effect of NCEs on neurobehavioural function (Redfern et al., 2005). The aforementioned behavioural assessments are not without their limitations, however, as this type of analysis is subjective and requires highly trained and experienced observers to ensure efficient reproducibility of experiments. Nonetheless, the simultaneous assessment of behaviour, locomotor activity, motor coordination and sensorimotor reflexes including nociception which are discussed below can be incorporated into a modified FOB (Redfern et al., 2005).

Locomotor activity and motor co-ordination

Procedures assessing locomotor activity generally rely on photoelectric beam interruption techniques using commercially available automated test systems, such as the Actimeter (Lynch et al., 2011). Although this methodology measures locomotion exclusively, assessment in conjunction with direct observational tests (e.g. modified Irwin test), can effectively determine whether a candidate drug has a sedative or psychostimulant effect by measuring the total distance covered in the cage (Lynch et al., 2011). Unlike behavioural experiments, these automated techniques are less labour intensive and allow the simultaneous investigation of an array of tests within a larger animal group (Porsolt et al., 2006). Therefore, data obtained from such techniques tend to be more statistically significant in comparison to data obtained by the subjective modified Irwin's test (Porsolt et al., 2006). Motor coordination

Table 3
Parameters assessed during safety pharmacology assessment of CNS. Table lists the various parameters assessed as part of the modified Irwin's test and the Functional Observation Battery (FOB) during CNS functional examination.
List modified from Redfern et al. (2005).

Autonomic nervous system	Sensorimotor	Neuromuscular	Behavioural
Salivation	Approach response	Posture	Arousal
Lacrimation	Grasping reflex	Gait	Vocalisation
Piloerection	Pupil response	Muscle tone	Handling reactivity
Excessive urination	Tail pinch/tail flick response	Grip strength	Stereotypy
Diarrhoea/loose faeces	Palpebral reflex	Tremor	Bizarre behaviour
Respiration	Startle/righting reflex	Shivering	Grooming
Rectal temperature	Landing foot splay	Convulsions	(Un)supported rears

function is most frequently assessed by the RotaRod method. Animals are trained on a rotating rod for a number of days prior to the first test session hence extensive training implemented by competent investigators is mandatory to ensure accuracy in assessments (Porsolt et al., 2006). This method directly investigates the effect of lead compounds on neuromuscular coordination, and thus, should be used in combination with other locomotor investigations to assess the overall effect on all aspects of motor function (Porsolt et al., 2006).

Sensorimotor reflexes and pain perception assessment

Identification of drug-induced gross defects in sensorimotor function is determined via manipulative neurological reflex examinations including pupil response, startle reflex and tail pinch, as illustrated in Table 2. These functional investigations are performed in a modified Irwin's test or FOB. In addition, using thermal and mechanical stimuli, nociception is assessed using a variety of basic techniques, such as, the hot plate, tail flick, paw pressure and plantar tests, which primarily record the latency of the nocifensive reflex response (Porsolt et al., 2002, 2006). This methodology is advantageous in its capacity to delineate analgesic properties of drugs as exemplified by morphine, which increases the time taken for the animal to react to noxious stimuli. Furthermore, this test can also be used to decipher whether a drug induces hyperresponsiveness to nocifensive stimuli (Porsolt et al., 2002).

CNS follow-up studies

Along with these core battery studies, the ICH has suggested non-mandatory additional studies to be performed during drug development (FDA, 2001). These investigations relate to higher cognitive function such as 'behavioural pharmacology, learning and memory, ligand-specific binding, neurochemistry, visual, auditory, and/or electrophysiology examinations' (FDA, 2001). Learning/memory paradigms used to assess cognition include the Morris maze and passive avoidance tests. These particular studies have been reviewed elsewhere (Porsolt et al., 2002). There is growing support for the requirement to perform more comprehensive CNS testing prior to FiH trials, including follow-up studies in proconvulsive activity and, more recently, drug abuse and dependence liability (Lindgren et al., 2008; Valentin et al., 2005).

Drug seizure liability

It is beneficial to investigate the proconvulsive activity associated with candidate drugs earlier in the drug development process in order to avoid future termination due to fatal drug-induced seizures, a major concern for the pharmaceutical industry. Drug seizure liability is generally assessed in rodent models, where convulsions are induced in the animal either by electrical stimulation across the cerebrum (electrocerebral silence (ECS) threshold test) or injection with the validated proconvulsant, pentylenetetrazol (PTZ seizure test) (Porsolt et al., 2006). Candidate drugs are administered prior to proconvulsive stimuli and their convulsive threshold with respect to the vehicle is determined (Porsolt et al., 2006). An increase and a decrease in seizure threshold are associated with anticonvulsive (as observed with phenobarbitol) and proconvulsive activity (as observed with D-amphetamine), respectively (Bankstahl et al., 2012). The ECS threshold test fails to deduce anticonvulsive activity, however, the PTZ seizure test can deduce both pro- and anticonvulsive activities (Porsolt et al., 2002). Nonetheless, it is important to note that both ECS and PTZ tests should be performed for full seizure liability assessment as discrepancies in both models have been documented (Bankstahl et al., 2012).

A more comprehensive method for assessing drug seizure liability is via electroencephalography (EEG), whereby implanted telemetric devices or electrodes fixed onto the brain surface measure brain electrical activity (Porsolt et al., 2006). This method is extremely sensitive in illustrating the proconvulsant activity of lead compounds where no overt convulsions are detected using the more traditional assessments. EEG can also assess drug induced convulsive effects on various regions of the brain. Seizure liability has been assessed via EEG in a variety of species, such as, non-human primates, dogs and rodents (Authier et al., 2009; Durmuller et al., 2007; Easter et al., 2007). Despite this, the EEG fails to provide mechanistic information on drug induced modulation of sensory receptors and their respective sensory motor pathways, illustrating a requirement for extensive in vitro molecular evaluation of targeted neuronal receptors (Porsolt et al., 2002).

Drug abuse and dependence liability

Commonly prescribed drugs, such as anxiolytic benzodiazepines (e.g. diazepam) and opioid painkillers (e.g. morphine), are frequently abused, due to their desirable psychotropic effects (Hernandez and Nelson, 2010). Such drugs can also induce physical and psychological side effects upon treatment cessation and thus are associated with human drug dependence (West and Gossop, 1994). Hence, preclinical evaluation of drug abuse and dependence liability of lead compound has become increasingly important in SP, with its inclusion in the regulatory guidelines by the European Medicines Authority (EMA, 2006) and the Food and Drug Administration (FDA, 2010).

Many initial in vitro and subsequent in vivo studies have been employed by pharmaceutical companies to evaluate the drug abuse and dependence liabilities of NCEs. The EMA and FDA have advocated a two-step evaluation of such studies. The initial tier relies on the comparison of lead compounds with established reference compounds of abuse, such as cocaine, using in vitro ligand binding, biogenic amine reuptake and synaptosomal dopamine release assays (Moser et al., 2011). Positive results from these studies are indicative of the NCE's risk abuse potential, and thus, must be confirmed in the second tier of in vivo drug abuse and dependence studies (Moser et al., 2011). These include investigations into the reinforcing properties of the drug (self-administration), the similarities of the psychotropic effects of the drug with known psychoactive compounds of abuse (drug discrimination) and its ability to cause unwanted physical/psychological effects upon drug withdrawal (i.e. drug dependence potential). Self-administration, drug discrimination and drug withdrawal tests are generally carried out in rodents, however, it has been debated that non-human primate models should also be used due to species differences in receptor profiles between rodent and humans (Ator and Griffiths, 2003).

During self-administration tests, rodents are trained to press a lever in order to self-administer an i.v. infusion of a known reference compound of abuse, such as cocaine (Moser et al., 2011). In a reinforcement schedule, the animal must execute a fixed number of operant responses in order to receive infusion of the positive 'rewarding' substance of abuse, also known as the fixed ratio (Moser et al., 2011). Subsequently, the reference compound is replaced with the test compound and the frequency at which the animal emits operant responses to receive the i.v. infusion of the test drug is indicative of its drug reinforcing properties and thus drug abuse potential (Moser et al., 2011). It is important to note that the sensitivity of this test is highly dependent upon the

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choice of training substance, thus validation with a variety of training substances should be implemented for greater accuracy of results. Unlike self-administration, drug discrimination procedures test the ability of the animal to distinguish between the subjective effects of a training drug of abuse to that of the vehicle (i.e. saline) using a two lever chamber (Moser et al., 2011). Drug discrimination is also highly specific in that the training drug must have a similar mechanism of action to the test compound (Glennon, 1999).

Unlike drug abuse, drug dependence is typified by observed physical and psychological withdrawal symptoms on drug treatment cessation, thus animal training is not required. Although many abused drugs are linked with drug dependence, such as morphine, heroin and alprazolam (Froger-Colleaux et al., 2011; Hernandez and Nelson, 2010), this does not necessarily mean that both drug abuse and dependence coincide with one another. Generally, rodents are chronically treated with the test drug over a 2–3 week period and withdrawal symptoms are evaluated over a week post drug treatment cessation (Moser et al., 2011). The EMA has listed the following as drug withdrawal endpoints: changes in behaviour, body temperature, body weight and food intake. Furthermore, it is suggested that multiple endpoints should be investigated to assess dependence liability, as no single measure is sufficient for complete evaluation. Additionally, the EMA recommends that observations should be made continually, over a long period of time (EMA, 2006).

An important point to consider when determining abuse and dependence liability, is the choice of species utilised (Moser et al., 2011). Preferential use of non-human primates over rodents has been suggested for specific assessment of the aforementioned parameters due to similarities in diurnality, drug metabolism and neurological receptor expression with humans (Moser et al., 2011).

Newer technology

New video automated testing systems, have been developed to evaluate visceral pain in rodents by quantifying licking behaviours in the rodent in response to a noxious stimuli (Hayashi et al., 2011). The neurokinin-1 receptor antagonist GR205171A was shown to potentiate licking responses associated with capsaicin administration (Hayashi et al., 2011). This automated method is high throughput and allows the quantification of licking behaviour over long periods of time. The emergence of integrated video EEG and computerized analysis has facilitated the simultaneous assessment of new compounds on behaviour (via video), seizure liability and disruption of sleep patterns (via EEG) in non-human primates (Authier et al., 2009). Therefore, continuous measurement with less interference is possible, giving an indication of long-term effects of the drug.

More recently, telemetry has been used in the continual assessment of withdrawal symptoms associated with morphine and chlordiazepoxide drug discontinuation in rats (Froger-Colleaux et al., 2011). It is worth noting that marked hypothermia and decreases in arterial blood pressure were observed in mice, 12 h after morphine discontinuation, during their nocturnal phase, thus highlighting the need for such automated technology in assessing drug dependence.

Respiratory system

Drugs of various pharmacological classes are known to have deleterious effects on respiratory functions including life threatening conditions (Murphy, 2002). More recently, drugs which had serious respiratory implications include Duragesic Patch and Advair. Prozac was another drug which increased the risk of pulmonary hypertension of the newborn in infants delivered by women who used Prozac during the third trimester of their pregnancy. Hence, a mandatory and detailed preclinical testing assessing the effects of new compounds on respiratory function was required. Therefore, as per the ICH recommendations, the SP assessment of the potential adverse reactions of new drugs requires evaluation of respiratory function as part of the core battery studies involving the vital organ systems (FDA, 2001). The guidelines indicate to carry out the two sets of studies, the core battery tests and follow-up studies. The core tests include the assessment of respiratory rate, tidal volume and haemoglobin oxygen saturation. Follow-up studies that are meant to provide greater depth of understanding of the core test observations include the assessment of airway resistance, compliance, pulmonary arterial pressure, blood gases and blood pH. The species used for routine testing based on the test compound and the study design include rodents, dogs and primates (Costa et al., 1992). However, special considerations on experimental design should be taken into account during species selection for respiratory safety testing which would improve the predictability of potential respiratory adverse events (Authier et al., 2008; Goineau et al., 2010).

Non-invasive plethysmography

The SP approach for assessing respiratory system involvement includes the assessment of pumping efficiency and gaseous exchange using a variety of measuring apparatus to assess these parameters (Table 4). Accurate ventilatory patterns are assessed to directly monitor lung volume changes or airflows generated by thoracic movements in conscious animals using a plethysmograph chamber (Adler et al., 2004; Hoymann, 2007; Murphy et al., 2010). Head-out, dual chamber and whole body plethysmography techniques are non-invasive methods that are currently used to evaluate typical parameters of respiration including tidal volume, minute volume, mid-expiratory flow, and respiratory rate (Gauvin et al., 2010). Industry opinion varies regarding the preferred method for preclinical safety assessment of respiratory function in the rat. A study which compared these three plethysmography methods in rodents reported that each system was equally sensitive. The whole body and head-out plethysmography provided consistent and reliable pulmonary mechanics data, while data collected from dual chamber plethysmography are clearly affected by restrainment stress in the animal (Gauvin et al., 2010). Recently, whole body and head-out plethysmography methods in conscious rats were compared, using

Table 4
Tests and parameters available to assess respiratory function in safety pharmacology studies. Table outlines the core and follow-up respiration associated parameters in SP testing. Also lists out the established and newer techniques associated with these investigations. PIF — Peak inspiratory flow; PEF — Peak expiratory flow; Ti — inspiratory time; Te — expiratory time; FIT — fractional inspiratory time; Penh — Enhanced pause.

Respiratory function assessment	
Readouts	
Core	Follow-up
Respiratory rate	Airway resistance
Tidal volume	Pulmonary arterial pressure
Haemoglobin oxygen saturation	Compliance
Established techniques	
Plethysmography	
Head out	
Tidal volume (VT); breathing rate (f); minute volume (VTxf); PIF/PEF/Ti/Te/FIT — in unrestrained animals	
Head out + pressure	
Tidal volume; breathing rate; minute volume; PIF/PEF/Ti/Te/FIT; compliance; resistance — in unrestrained animals	
Head-enclosed	
Tidal volume; breathing rate; minute volume; PIF/PEF/Ti/Te/FIT; specific airway resistance — in restrained animals	
Barometric whole body	
Tidal volume; breathing rate; minute volume; FIT; Penh	
By induction/impedance	
Telemetry (external/Implanted) — tidal volume; breathing rate; minute volume	
Invasive	
Pulmonary resistance and compliance	
Emerging techniques	
Unrestrained video-assisted plethysmography	
Telemetry	
Biomarkers: VQM — Ventilation (V)/perfusion (Q) mismatch (M)	

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theophylline as a respiratory stimulant and chlordiazepoxide as a respiratory depressant. The study reported that respiratory function can be accurately evaluated using head-out plethysmography compared to whole body plethysmography. The authors also addressed the demand for additional invasive methods to evaluate ventilator parameters such as mid-expiratory flow (Nirogi et al., 2012). Another non-invasive respiratory function assessment is the use of the variable, enhanced pause (Penh), which is measured by whole body plethysmography (barometric) in unrestrained animals. Despite being a simple procedure, it was found that Penh was less reliable compared to head-out plethysmography method in its correlation with other pulmonary parameters such as resistance, hence is not used extensively as part of respiratory SP core battery studies (Hoymann, 2012). Thus, non-invasive whole body or head-out plethysmography is the most common system used to evaluate the ventilatory function in conscious animals in the laboratory. Non-invasive head-out body plethysmography measurements for core battery respiratory SP studies in conscious rodents are reliable, as it is simple to handle, the breathing pattern is nearly natural (anaesthesia is not required) and it allows high-throughput screening. Training the animals in the chamber prior to experimentation will reduce the animal stress induced variation in the assessments. However, lung resistance and compliance assessments to refine respiratory SP profile cannot be obtained using head-out or whole body plethysmography.

Invasive plethysmography

Follow-up respiratory SP studies using invasive plethysmography methods are performed to further investigate any unwanted potentially deleterious effects on respiratory functions observed during core battery studies, or any potential adverse effects that may be suspected due to the inherent pharmacological properties of the test compound. These studies involve the assessment of changes in the mechanical properties of lungs such as pulmonary resistance and compliance for the identification of bronchoconstriction and obstruction. Invasive procedures designed to assess these parameters accurately involves orotracheal intubation, pulmonary manoeuvres and surgical implantation of pleural pressure sensors for chronic resistance recording or tracheotomised, intubated animals (Hoymann, 2012). The advantages of these techniques are that they do not factor restrainment stress of animals in the measurements and are accepted as the gold standard for accurate assessment of resistance and compliance. The major drawbacks include the use of anaesthesia which decreases the breathing frequency and the requirement of experienced and specially trained personnel.

Newer technology

Similar to the other SP vital organ studies, telemetry can also be used effectively in respiratory safety assessment (Delaunois et al., 2009). The Kearney group has evaluated a novel surgical implanted telemetry method incorporated with an impedance sensor for chronic evaluation of respiratory parameters (Kearney et al., 2010). They validated the use of such implantable telemetry via successful comparison with pneumotachograph recorded values in conscious Beagle dogs following i.v. administration of doxapram. This type of technology has also been validated in non-human primates allowing the simultaneous evaluation of both CVS and respiratory function (Authier et al., 2010). Another variant in this technology is the use of respiratory inductive plethysmography (RIP) with telemetry which allows the continuous monitoring of respiratory parameters in non-restrained large animals for extended periods of time including awake and sleep states (Murphy et al., 2010). All these experimental approaches are dedicated to ventilatory machinery (the pumping apparatus) rather than to a true evaluation of respiration efficiency. In this respect, blood gas analysis and haemoglobin saturation should not be neglected. Newer and emerging approaches for respiratory SP include modifications in plethysmography, telemetry and potential biomarkers for specific respiratory disorder. Barometric, whole-body plethysmography is a safe, non-invasive and reliable technique for investigation of lung function in dogs which provides new opportunities to characterise respiratory status (Talavera et al., 2006). Unrestrained video-assisted plethysmography is an emerging approach which can be performed in small animals, such as rodents, to assess specific airway resistance and the breathing pattern, accurately, in a non-invasive fashion (Bates et al., 2008).

Ventilation (V)/perfusion (Q) mismatch (VQM) is the main cause of gas-exchange abnormalities observed in various pulmonary diseases. It can be exacerbated by certain pharmacological agents resulting in unwanted effects on the respiratory system, including hypoxemia. A recent report has addressed the relevance, techniques to assess VQM, and the potential use of VQM as a safety biomarker during drug development (Amen et al., 2011). With further validation, VQM can be used in respiratory SP based on the pharmacological properties of the NCE being explored for development.

Supplemental organ systems and studies

Gastrointestinal system

Gastrointestinal (GI) complications are common side effects, with varying degrees of severity, observed during and after drug development, and are associated with drug-induced morbidity (Pirmohamed et al., 2004). Drug induced GI complications include nausea, emesis, constipation and may also affect the absorption of other drugs. Therefore, it is important to study the effect of the test drug on the GI system (Harrison et al., 2004), routinely, to improve the safety and efficacy for NCE development. According to ICH S7A recommendations, the effect of test compounds ought to be assessed using gastric emptying, intestinal motility and gastric secretion in appropriate animal models. Evaluation of GI function is supplementary and, therefore, is indicated based on the knowledge of the NCE being tested (FDA, 2001; Harrison et al., 2004). The commonly altered GI physiological functions include motility and ulcerations, but also gastric mucus production, hydrochloric acid and bicarbonate secretion, which are commonly seen with prostaglandin E1 analogues and some non-steroidal anti-inflammatory drugs (NSAIDs). The effects of test compounds on the GI system are commonly evaluated in rodent models, using tests assessing: gastric emptying, intestinal motility, gastric secretion and GI injury (Harrison et al., 2004). The SP tests available to assess drug-induced GI changes are shown in Table 5.

Gastric emptying and intestinal motility

Gastric emptying and intestinal motility is evaluated by feeding the animals with barium sulphate ($BaSO_4$) or a charcoal test meal subsequent to test compound administration. The test meals may be used either as an indicator for liquid transport (phenol red) or for transport of solids ($BaSO_4$, charcoal). At the desired time point, ideally close to C_{max}, the stomach is extracted and weighed, since the weight

Table 5
Tests and parameters available to assess gastrointestinal function and integrity in safety pharmacology studies. Table lists both established and newer techniques in gastrointestinal SP studies. EMG — electromyograph; miR — microRNA; PBPK — physiologically based pharmacokinetics.

Gastrointestinal toxicity assessment	
Function	Injury
Established	
Gastric emptying	Macroscopic (ulcer index)
Intestinal motility	Histopathology
Gastric secretion	
Emerging	
Endoscopy	Endoscopy
Capsule — pH, pressure	Capsule
Radiotelemetry	Biomarkers
Strain gauges for contraction, EMG	Citrulline
In-silico (PBPK modelling)	miR-194
	Calprotectin

of the stomach is directly correlated to the weight of the gastric content. Weighing of the stomach when full and empty for stomach content weight is necessary to obtain more reliable results. Changes in the weight between the test groups indicate altered gastric emptying. Regarding intestinal motility measurements, intestines from the duodenum (to either ileum or rectum) are prepared, and the length of the intestine filled with $BaSO_4$ or charcoal from the test meal in relation to the length of the whole gut is determined by visual inspection. Any difference in the $BaSO_4$/charcoal transit length between the test groups and the controls infer alteration in the intestinal motility. When phenol red is used, any change in the spectral absorbance in specific parts of the gut (normally collected in ten sub segments) indicates altered intestinal transit.

Gastric secretion

Gastric secretion is evaluated by the parenteral administration of the test drug following pylorus ligation and the stomach contents act as screen for changes, which only occur locally, in volume, pH, total acidity and acid output over time. Gastric secretion tests are typically performed following changes in gastric emptying. Agonists of opioid, dopamine receptors, and beta-adrenoceptors markedly reduce gastric emptying and intestinal motility. However, muscarinic receptor agonists tend to increase gastric emptying, intestinal motility, and gastric secretion, whereas antagonists have the opposite effects. Unpublished data from Dr Sabine Pestel (*Boehringer-Ingelheim Pharma GmbH & Co*) on 59 test compounds evaluated between 2009 and 2001 showed a greater incidence and severity on gastric emptying (85% vs. 45%) and intestinal transit (70% vs. 25%) of compounds derived from oncology vs. non-oncology projects. Those effects were detected at lower margins for oncology vs. non-oncology projects (~2–5 vs ~10–30-fold on a dose basis). It is important to note that anticancer compounds have shown greater GI complications hence it would be beneficial to include GI testing as part of the routine safety pharmacology studies for this class of compounds.

Newer technology

GI injury assessments are usually performed following lead candidate drug administration and are preformed through visual examination of the stomach and intestinal tract and ulceration index scores. A recent advance in SP for GI assessment is the use of biomarkers for GI injury. Biomarkers specific for GI injury, such as blood citrulline, faecal miR-194 and calprotectin, are being explored and hold promise in safety assessments (John-Baptiste et al., 2012). However, further validation and consensus are needed prior to their implementation in routine SP assessments. In addition, the use of the wireless capsule, radiotelemetry and in-silico (PBPK modelling) in the assessment and prediction of gastric emptying, intestinal motility and GI injury to reduce undue stress to the animals and to reduce animal numbers are also being explored.

Renal system

Based on the data available from preclinical testing and clinical trials, it can be inferred that drug-induced changes in kidney function, including nephrotoxicity, may be underestimated (Fuchs and Hewitt, 2011; Fung et al., 2001; Pirmohamed et al., 2004). In addition, unpublished data from Dr Sabine Pestel (*Boehringer-Ingelheim Pharma GmbH & Co*) on 99 test compounds evaluated between 2004 and 2011 showed that nearly 70% of all test compounds demonstrated effects on renal function, and close to 50% were indicative of kidney injury based on changes in the biomarkers. Therefore, there is a growing need to integrate routine evaluation of the renal system into SP testing, which can be grouped into altered renal functions (diuresis or anti-diuresis) and organ damage, such as acute kidney injury (AKI), this can include localized injury to glomeruli, renal papillae and/or different regions of the tubules (Lienemann et al., 2008). According to ICH recommendations, testing of renal function by measuring urine volume and electrolyte excretion in rats or dogs, as part of SP, is supplementary or is indicated based on the knowledge obtained about the NCE under test (FDA, 2001).

Routinely, clinical chemistry-based evaluations, using urine and serum samples, are used to assess drug-induced renal impairment (Pestel et al., 2006, 2007; Pugsley et al., 2008) and isolated organ preparations are carried out for additional mechanistic studies. Table 6 summarizes the various approaches and parameters in the renal SP testing. A report can be referred for objective analysis of renal assessment strategies in SP (Emeigh Hart, 2005). The battery of tests includes measurement clearance rate, glomerular filtration rate (GFR), urinary volume, osmolality, pH, Na^+, Cl^-, K^+, creatinine and urea, along with serum Na^+, Cl^-, K^+, creatinine and BUN (blood urea nitrogen) for assessment of kidney function.

Renal function assessments

GFR, a main parameter for assessing renal function is calculated using both urine and serum samples obtained from the animals. Multiple serum collections should not be taken before/during urine sampling, as blood sampling will affect urinary volume (Stonard, 1990). Nevertheless, it would be useful to have samples from multiple time points, since knowledge of kinetics is necessary to understand the function. Hence, limiting to three samples within 24 h would prove beneficial without causing any other interference (Pestel et al., 2007). Therefore, mathematical modelling is used to extrapolate the data obtained to calculate GFR and improve the reliability of the data, while using fewer animals (Pestel et al., 2007). If the study design requires samples from the same animal, larger animals, such as dogs, can be used (Rahma et al., 2001). However, an integrated pharmacology testing system in surgically prepared rats has been recently developed for simultaneous measurements of GFR and renal plasma flow. This system successfully combined BASi Culex® automated blood sampling, radiotelemetry, quantitative urinalysis, and nephron site-specific urinary biomarkers of injury into one model testing system (Kamendi et al., 2010; Litwin et al., 2011). Renal toxicity can be predicted using clinical chemistry following a single administration of the test drug (Pestel et al., 2006),

Table 6
Parameters to assess renal function and integrity of the kidney in safety pharmacology investigations. Table lists both established and emerging parameters in renal safety pharmacology studies. ALP — alkaline phosphatase; AST — aspartate aminotransferase; ALT — alanine aminotransferase; BUN — blood urea nitrogen; CLU — clusterin; GGT — γ-glutamyl transferase; GFR — glomerular filtration rate; GST — glutathione S transferase; KIM-1 — kidney injury molecule-1; LDH — lactate dehydrogenase; β-NAG — *N*-acetyl-β-*D*-glucosaminidase; NGAL — neutrophil gelatinase-associated lipocalin; RPA-1 — renal papillary antigen-1; TFF3 — trefoil factor 3.

Renal function	Renal injury markers			
	Qualified (known)		Qualified leakage markers (New)	New leakage/ inducible markers under investigation
	Functional makers	Leakage markers		
Urine	Urine	Urine	KIM-1	Glomerulus
Volume	Glucose	AST	CLU	Albumin
Osmolality	Protein	ALT	TFF3	Cystatin C
pH	Albumin	LDH		β2-microglobulin
Na^+	Creatinine	GGT		Total protein
Cl^-	Urea	ALP		Proximal tubule
K^+	Cystatin C	β-NAG		α-GST
Serum	β2-microglobulin (new)			KIM-1
Na^+	Serum			NGAL
Cl^-	Creatinine			Cystatin C
K^+	BUN			CLU
Calculated	Cystatin C			β2-microglobulin
Clearance rate				TFF3
GFR				Distal tubule
				μ-GST
				Collecting duct
				RPA-1
	Histopathological markers			

but the sensitivity is rather low when compared to NMR-based metabolomics methods (Lienemann et al., 2008). However, with newer evaluation tools and semi-automatic approaches, sensitivity could be considerably increased.

Kidney injury markers

Kidney injuries are also being assessed using functional and leakage markers. Functional markers suggesting kidney injury may include urinary glucose, protein, albumin and calciumor, indeed, any other molecule known to be transported in a certain region of the kidney. Urinary excretion of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP) and *N*-acetyl-β-*D*-glucosaminidase (β-NAG) are used as leakage markers for kidney injury measured by clinical chemistry. Further leakage markers like kidney injury molecule-1 (KIM-1) and clusterin (CLU) can be measured with different techniques based on antibody detection. Acute kidney injury (AKI) predominantly includes proximal tubule toxicity due to the high concentration of test drug in the loop of Henle and renal papillae, injuries here are more commonly associated with drug-induced nephrotoxicity (Miller, 2002). These kidney injuries are assessed primarily using histology and approved biomarkers. In rats, drug toxicity has been shown to vary with circadian rhythm application (Levi et al., 1982), since kidney functions are shown to be influenced significantly by time of day (Globig et al., 1999; Pons et al., 1996). The various parameters both established and emerging in renal SP studies are shown in Table 6.

Newer technology

One of the recent advances in SP which can increase the depth and breadth of renal toxicity (functional & injury) assessments, is the use of molecular biomarkers. The use of molecular biomarkers improves the predictability of renal toxicity as histological examination can contribute to false negative findings, due to the time taken for histopathological manifestation following insult, and region of section used for the examination (regional bias). Therefore, there is a need for molecular biomarkers to detect and predict region specific nephrotoxicity more effectively (Muller and Dieterle, 2009) Recently, newer kidney injury biomarkers qualified for preclinical testing include KIM-1, CLU, albumin, total protein, β2-microglobulin, cystatin C and trefoil factor 3 (TFF3) in urine (Dieterle et al., 2010). Some of these biomarkers can provide key information on the region of injury as indicated in Table 6. Owing to the potential of molecular biomarkers in contributing to false positive findings, a positive association in predicting renal toxicity should be based on information obtained collectively from renal function assessment, histology and molecular biomarker readout. Recently, metabolomics approaches involving the use of NMR and mass spectroscopy to identify known nephrotoxic biomarkers are being explored (Boudonck et al., 2009; Lienemann et al., 2008).

Recent and emerging concepts

SP is continuously evolving and some recent trends to enhance and refine the scope include focus towards frontloading, exploration of alternate models, combining core battery tests, integration of SP endpoints into regulatory toxicology endpoints and correlation between non-clinical safety endpoints and clinical outcomes. As techniques and methodologies continue to improve, SP has adapted to contribute to improved decision making in lead candidate selection during drug discovery and development.

Frontloading

There is a clear need for the implementation of safety assessments in the initial stages of drug discovery and development which would facilitate ranking of NCEs leading to the improved identification of lead candidates, ultimately reducing valuable time and costs involved in the drug discovery and development process. This requirement is addressed by the practice of "frontloading" in SP studies. "Frontloading" is defined as "safety studies conducted during lead optimisation of compounds before selection of a candidate drug for development and regulatory studies are performed (Lindgren et al., 2008). Understanding more about the propensity of molecules to cause adverse effects prior to initiation of in vivo studies is becoming increasingly important to reduce the likelihood of termination at later stages of drug development. Unlike the core battery assessments, frontloading SP studies are not performed according to GLP compliance (Lindgren et al., 2008). The current practice and perspective of frontloading in major organ system SP assessment have been discussed elsewhere (Lindgren et al., 2008).

With regard to the CVS, this challenge can be tackled by performing in vitro assays, similar to the hERG assay, for many of the ion channels previously mentioned. Furthermore, telemetry studies can also be used to provide in vivo assessment for numerous NCEs' effects on the CVS, prior to pre-clinical trials. From a CNS safety pharmacology perspective, in vitro receptor ligand binding assays are used to assess potential NCE-induced effects on a variety of neuronal targets including gamma-aminobutyric (GABA), N-Methyl-*D*-aspartic acid (NMDA) and dopamine receptors which have been extensively reviewed elsewhere (Bowes et al., 2012). Frontloading can also be applied to assess seizure liability through in vitro assays, such as the semi-automated Slicemaster system, that only requires minute concentrations of the NCE and can measure electrophysiological recordings in up to eight rodent hippocampal brain slices (Easter et al., 2007). However, they can only assess pro-convulsive activity in specific brain regions and since seizures have a complex mechanism these assays should be complemented with in vivo assessment. Frontloading in respiratory SP studies include selectivity binding screens, rodent plethysmography and arterial blood gas measurement which are the common techniques used, whereas isolated organs/tissues/cells and anaesthetized animals are used if there is a need to assess lung mechanics as part of frontloading (Lindgren et al., 2008). For the renal system, routine practice of frontloading is relatively low (Lindgren et al., 2008; Pugsley et al., 2008); the same holds true of the GI system.

Taken together, the frontloading concept not only facilitates the early identification of potentially hazardous substances, thus contributing to better decision making for the selection of safer candidate drugs administered in FiH trials, but also reduces the number of in vivo safety studies required to decipher the toxicity of such NCEs as a result of early termination of potentially unsafe candidates.

Alternate models

The zebrafish is a well-established model organism for use in developmental biology and more recently in toxicology and disease (Ali et al., 2011; McGrath and Li, 2008). The zebrafish model in CNS studies has been validated, offering a 'sufficient', 72% predictability of proconvulsive activity through the use of validated anticonvulsant and proconvulsant compounds in assessing seizure liability, via automated measurements of locomotor activity (Winter et al., 2008). Similarly to the in vitro hippocampal brain slice assay, relatively small amounts of NCEs are required to perform the screen. Many other behavioural paradigms, such as addiction, memory and anxiety can be assessed using the zebrafish model (Ninkovic and Bally-Cuif, 2006). There is a great potential for this model to be used in early drug fail fast strategies, especially for CNS targeted NCEs. Renal safety assessment studies conducted in simpler animal models and/or simple organs, such as teleost pronephros systems in zebrafish, can render renal safety testing routine (Barros et al., 2008; Redfern et al., 2008). This model can be explored as one of the more viable options, without compromising on the predictability of adverse events, since, its gentamicin-induced patho-phenotype was similar to that of those observed in the mammalian renal system. However, the use of in vivo zebrafish models as early screening methods in SP is a matter of debate

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(Barros et al., 2008) as the use of this model is yet to be recognised by the regulatory bodies. Nonetheless, it is amenable to the early phases of drug discovery in terms of turnaround time, cost, NCE requirement and throughput, thus having the potential to facilitate early screening methods in screening for safety liabilities (Barros et al., 2008).

The exploitation of human embryonic stem cell derived cardiomyocytes (hESC-CM) and human inducible pluripotent stem cell derived cardiomyocytes (hiPS-CM) as models of in vitro high throughput drug screening and CVS safety assessment have the potential to significantly refine CVS studies due to their biological relevance and mass production capacity (Kraushaar et al., 2012). Unlike mammalian cell lines, these cells inherently express the hERG channel and other ion channels which contribute to the AP as well. As heart complications are not simply due to hERG blockade alone, these cell lines can facilitate the measurement of a multitude of target ion channels, thus enhancing the SP profile of NCEs (Kraushaar et al., 2012). hESC-CMs, have shown to be more sensitive compared to current in vitro isolated tissue preparations in CVS safety assessments (Peng et al., 2010). Importantly, iPS-CMs derived from patients with long QT syndrome emulated the electrophysiological features of the disorder revealing the irrefutable potential of the use of iPS-CMs derived from human patients for utilisation of drug screening in appropriate disease models (Moretti et al., 2010). Therefore, this approach will offer the opportunity to screen not only NCEs in normal tissue but also hiPS-CM originating from patients suffering various disease(s), offering a disease-model approach focused on the anticipated target population. Although these models are promising, they are not without their shortcomings as issues with stability of cardiomyocyte phenotype, genetic variation and reproducibility of differentiation remain a concern. Thus, these models require further validation and standardisation in order to be fully implemented in CVS SP studies (Kraushaar et al., 2012).

Integrated core battery assessment

As mentioned previously, telemetry, an increasingly popular technique, is evolving to provide relevant and reliable in vivo data from a variety of physiological systems that are examined as part of SP studies. This revolutionary technique has changed SP so that many core battery safety studies, which are traditionally investigated separately, can now be measured simultaneously in conscious animals across a variety of species (Moscardo et al., 2010; Tontodonati et al., 2007). This not only reduces the number of animals used per study, but also enhances the statistical power of the results as the animals can be used as their own respective vehicle control (Tontodonati et al., 2007). A prime example of this is the use of integrated video telemetry in assessing the neurobehavioural (via video recordings) and cardiovascular (via telemetric devices) effects of candidate drugs in canine and non-human primate models (Moscardo et al., 2010; Tontodonati et al., 2007). Combining video recording with telemetry allows integrated CNS and CVS observations over extended periods of time with minimal stress caused to experimental animals. The combination of respiratory SP studies using radio telemetry and automated blood sampling offers an integrative pharmacological and toxicological approach inevitably decreasing the number of animals without compromising, the credibility of the data obtained and the predictive ability of the studies (Kamendi et al., 2010). The use of emerging technologies will aid in the integration of GI toxicity screening as part of the other mandatory core testing, since methods like capsule endoscopy and radio-telemetry are non or less invasive and can be used simultaneously alongside cardiovascular and respiratory assessments (Gacsalyi et al., 2000; Kramer and Kinter, 2003).

Integrating safety pharmacology end points into toxicology studies

SP can be referred to as studies that investigate the possible undesirable pharmacodynamic effects on physiological functions as a result of exposure to the compound in the therapeutic range and above, before evaluating and investigating the cause of these effects through toxicological and/or clinical studies (Valentin and Hammond, 2008). On the other hand, toxicological studies focus on exploring the adverse pharmacodynamic effects of compounds up to the maximum tolerated dose level. In particular, they centre on addressing general safety and are designed to include high doses at which overt toxicity may be observed (Guth et al., 2009). Integration of SP and toxicology studies will improve the resolution of the safety profile and risk factor identification more effectively (Claude and Claude, 2004; Luft and Bode, 2002). When integrating SP and toxicological studies, consideration needs to be given to various factors: the selection of species, number of animals, study designs, reduction of cost and timelines to the endpoints that can be integrated (Luft and Bode, 2002; Pugsley et al., 2008). SP studies are typically single-dose studies in which a given effect can be measured over time, while in toxicological studies, data may be collected sequentially over days or weeks of treatment, especially for substances that may chronically accumulate in the body (Guth et al., 2009). Personnel training is essential for effective integration of SP and toxicological end point assessments (Valentin and Hammond, 2008). Additionally, animals have to be trained in order to reduce stress level during routine sample collection and care should be also be taken to avoid disturbances to the animals which may disrupt physiological functions and SP read outs (Bass et al., 2004). Sometimes there is no viable solution and multiple experiments do need to be performed, but with careful planning and compromise, this can normally be accomplished, as a well-designed SP study could allow for multiple administrations of a compound (Guth et al., 2009). With regards combining SP and toxicology in CNS, behavioural tests such as the modified Irwin test or FOB can be easily integrated into toxicology studies with minimal or no impact on histological data obtained (Luft and Bode, 2002). The main disadvantage when combining behavioural assessments and toxicology studies is that the data received can be highly influenced by the experience and training of the individuals which perform and interpret the assessments as indicated earlier. Another important issue when combining these endpoints is that the behavioural assessments need to be conducted when other parameters, such as blood sampling are not being measured; this avoids the possibility of sampling affecting the other parameters. However, in long-term toxicology studies this should not be an issue as long as there is good communication between the personnel performing the behavioural assessments and toxicology studies. Currently, there are guidelines (ICH S6, ICH S7A and ICH S9) that relate to the integration of SP endpoints in toxicology studies and this will become more prevalent in the future.

Drug–drug interactions

As mentioned earlier in this review, drug–drug interactions can cause adverse side effects that can lead to attrition of lead candidates or drugs. There are a number of assays available to assess the binding properties of an NCE (Kramer et al., 2007) and these include the extent of cytochrome P450 inhibition (Wienkers and Heath, 2005) and P-glycoprotein interactions (Hollo et al., 1994). In vitro binding affinities should be used cautiously when extrapolating in vivo data; however, with well-designed experiments these assays can provide benefits with regard to compound design and the prediction of potential unwanted interactions. Given the low cost of these assays, it would be beneficial to include these preliminary screens and this is supported by the recent ICH draft guidance (EMA, 2013).

Translational safety pharmacology

SP is evolving to keep pace, adapt, to incorporate the latest scientific knowledge and novel technologies for the safety evaluation of compounds in non-clinical assays, and to identify the effects that may pose a risk to human volunteers and patients. There are recent

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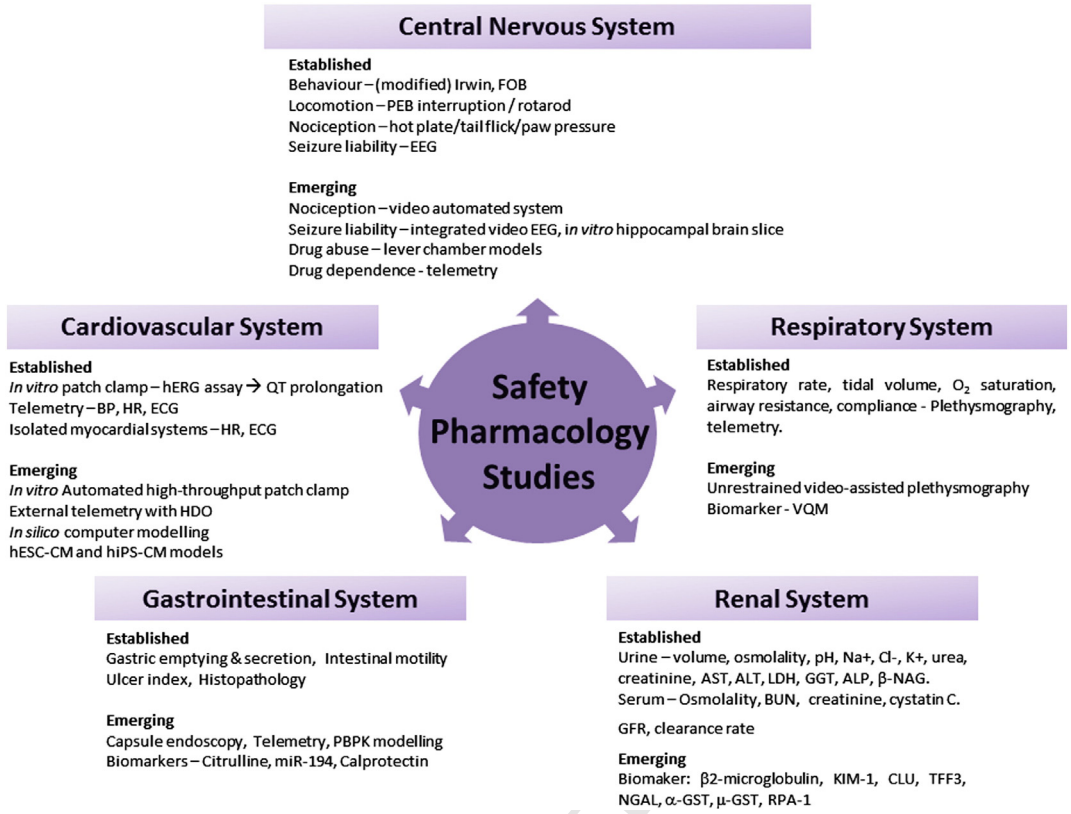

Fig. 2. Established and emerging parameters and techniques in safety pharmacology studies. Illustration established and emerging parameters/techniques investigated in five important organ systems to assess lead compounds in safety pharmacology studies. AP — action potential; ALP — alkaline phosphatase; AST — aspartate aminotransferase; ALT — alanine aminotransferase; BP — blood pressure; BUN — blood urea nitrogen; CLU — clusterin; CM — cardiomyocyte; EEG — electroencephalography; ECG — electrocardiogram; FOB — Functional Observation Battery; GGT — γ-glutamyl transferase; GFR — glomerular filtration rate; GST — glutathione S transferase; HDO — high definition oscillometry; hESC-CM — human embryonic stem cell derived cardiomyocytes; hiPS-CM — human inducible pluripotent stem cell derived cardiomycotes; HR — heart rate; KIM-1 — kidney injury molecule-1; LDH — lactate dehydrogenase; miR — microRNA; β-NAG — *N*-acetyl-β-*D*-glucosaminidase; NGAL — neutrophil gelatinase-associated lipocalin; PBPK — physiologically based pharmacokinetics; PEB — photoelectric beam interruption technique; RPA-1 — renal papillary antigen-1; TFF3 — trefoil factor 3; VQM — ventilation (V)/perfusion (Q) mismatch (M).

examples of promising future areas for the development of SP that illustrate the challenges, as reviewed in (Bass et al., 2011; Valentin and Hammond, 2008). However, a more sophisticated translation of human outcomes to preclinical animal models and vice versa still remains an essential goal. Several individual organisations or consortia efforts are trying to address this issue by conducting retrospective analysis (Trepakova et al., 2009; Valentin et al., 2009; Wallis, 2010) or prospective studies (Lawrence et al., 2006; Winter et al., 2008). It is clear that the confidence in the translational SP models will improve as the number of NCEs that progress through SP model and subsequent human trials increases. This will enhance the validity of the non-clinical safety assessment models that are used ultimately facilitating better decision making at all stages of drug discovery and development.

Summary

Over the last decade, SP has made tremendous progress in both the regulatory requirements and the knowledge gained while developing NCEs (Bass et al., 2011). A schematic summation of the current and emerging trends in SP studies is represented in Fig. 2. It has become increasingly evident that more suitable high throughput in vitro screening methods are required to be implemented at the earliest stages of drug discovery to obtain information about compounds prior to the initiation of clinical trials. Fail fast drug strategies at this stage would prevent the progression of potential unsafe NCEs into later discovery, thus saving valuable time and costs for the pharmaceutical industry. It is also worth exploring the value of using other models to answer various SP questions. Although the emergence of the zebrafish model is still a matter of debate, the zebrafish lends real potential as a fast means for early compound screening in all aspects of frontloading (Barros et al., 2008). Further validation of this model in a variety of studies may result in their regular use as a frontloading model in the future. The incorporation of the emerging concepts, such as biomarkers and common SP-toxicological endpoints, should be carried out alongside mandatory SP protocols to validate the accuracy and reproducibility of these tests, which will ultimately augment SP studies and predictive end points for safer therapeutics.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

Adler, A., Cieslewicz, G., Irvin, C.G., 2004. Unrestrained plethysmography is an unreliable measure of airway responsiveness in BALB/c and C57BL/6 mice. J. Appl. Physiol. 97, 286–292.

Ali, S., Champagne, D.L., Spaink, H.P., Richardson, M.K., 2011. Zebrafish embryos and larvae: a new generation of disease models and drug screens. Birth Defects Res. C Embryo Today 93, 115–133.

Amen, E.M., Becker, E.M., Truebel, H., 2011. Analysis of V/Q-matching—a safety "biomarker" in pulmonary drug development? Biomarkers 16 (Suppl. 1), S5–S10.

Ator, N.A., Griffiths, R.R., 2003. Principles of drug abuse liability assessment in laboratory animals. Drug Alcohol Depend. 70, S55–S72.

Authier, S., Legaspi, M., Gauvin, D., Chaurand, F., Fournier, S., Troncy, E., 2008. Validation of respiratory safety pharmacology models: conscious and anesthetized beagle dogs. J. Pharmacol. Toxicol. Methods 57, 52–60.

Authier, S., Paquette, D., Gauvin, D., Sammut, V., Fournier, S., Chaurand, F., Troncy, E., 2009. Video-electroencephalography in conscious non human primate using radiotelemetry and computerized analysis: refinement of a safety pharmacology model. J. Pharmacol. Toxicol. Methods 60, 88–93.

Authier, S., Haefner, P., Fournier, S., Troncy, E., Moon, L.B., 2010. Combined cardiopulmonary assessments with implantable telemetry device in conscious freely moving cynomolgus monkeys. J. Pharmacol. Toxicol. Methods 62, 6–11.

Bankstahl, M., Bankstahl, J.P., Bloms-Funke, P., Loscher, W., 2012. Striking differences in proconvulsant-induced alterations of seizure threshold in two rat models. Neurotoxicology 33, 127–137.

Barros, T.P., Alderton, W.K., Reynolds, H.M., Roach, A.G., Berghmans, S., 2008. Zebrafish: an emerging technology for in vivo pharmacological assessment to identify potential safety liabilities in early drug discovery. Br. J. Pharmacol. 154, 1400–1413.

Bass, A.S., Vargas, H.M., Kinter, L.B., 2004. Introduction to nonclinical safety pharmacology and the safety pharmacology society. J. Pharmacol. Toxicol. Methods 49, 141–144.

Bass, A.S., Vargas, H.M., Valentin, J.P., Kinter, L.B., Hammond, T., Wallis, R., Siegl, P.K., Yamamoto, K., 2011. Safety pharmacology in 2010 and beyond: survey of significant events of the past 10 years and a roadmap to the immediate-, intermediate- and long-term future in recognition of the tenth anniversary of the Safety Pharmacology Society. J. Pharmacol. Toxicol. Methods 64, 7–15.

Bates, J.H., Thompson-Figueroa, J., Lundblad, L.K., Irvin, C.G., 2008. Unrestrained video-assisted plethysmography: a noninvasive method for assessment of lung mechanical function in small animals. J. Appl. Physiol. 104, 253–261.

Boudonck, K.J., Rose, D.J., Karoly, E.D., Lee, D.P., Lawton, K.A., Lapinskas, P.J., 2009. Metabolomics for early detection of drug-induced kidney injury: review of the current status. Bioanalysis 1, 1645–1663.

Bowes, J., Brown, A.J., Hamon, J., Jarolimek, W., Sridhar, A., Waldron, G., Whitebread, S., 2012. Reducing safety-related drug attrition: the use of in vitro pharmacological profiling. Nat. Rev. Drug Discov. 11, 909–922.

Claude, J.R., Claude, N., 2004. Safety pharmacology: an essential interface of pharmacology and toxicology in the non-clinical assessment of new pharmaceuticals. Toxicol. Lett. 151, 25–28.

Costa, D.L., R.J.A., Tepper, J.S., 1992. Comparative respiratory physiology of the lung. CRC press, Boca Raton.

Couderc, J.P., Xiaojuan, X., Zareba, W., Moss, A.J., 2005. Assessment of the stability of the individual-based correction of QT interval for heart rate. Ann. Noninvasive Electrocardiol. 10, 25–34.

Curran, M.E., Splawski, I., Timothy, K.W., Vincent, G.M., Green, E.D., Keating, M.T., 1995. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell 80, 795–803.

Delaunois, A., Dedoncker, P., Hanon, E., Guyaux, M., 2009. Repeated assessment of cardiovascular and respiratory functions using combined telemetry and whole-body plethysmography in the rat. J. Pharmacol. Toxicol. Methods 60, 117–129.

Dieterle, F., Sistare, F., Goodsaid, F., Papaluca, M., Ozer, J.S., Webb, C.P., Baer, W., Senagore, A., Schipper, M.J., Vonderscher, J., Sultana, S., Gerhold, D.L., Phillips, J.A., Maurer, G., Carl, K., Laurie, D., Harpur, E., Sonee, M., Ennulat, D., Holder, D., Andrews-Cleavenger, D., Gu, Y.Z., Thompson, K.L., Goering, P.L., Vidal, J.M., Abadie, E., Maciulaitis, R., Jacobson-Kram, D., Defelice, A.F., Hausner, E.A., Blank, M., Thompson, A., Harlow, P., Throckmorton, D., Xiao, S., Xu, N., Taylor, W., Vamvakas, S., Flamion, B., Lima, B.S., Kasper, P., Pasanen, M., Prasad, K., Troth, S., Bounous, D., Robinson-Gravatt, D., Betton, G., Davis, M.A., Akunda, J., McDuffie, J.E., Suter, L., Obert, L., Guffroy, M., Pinches, M., Jayadev, S., Blomme, E.A., Beushausen, S.A., Barlow, V.G., Collins, N., Waring, J., Honor, D., Snook, S., Lee, J., Rossi, P., Walker, E., Mattes, W., 2010. Renal biomarker qualification submission: a dialog between the FDA-EMEA and Predictive Safety Testing Consortium. Nat. Biotechnol. 28, 455–462.

Dunlop, J., Bowlby, M., Peri, R., Vasilyev, D., Arias, R., 2008. High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiology. Nat. Rev. Drug Discov. 7, 358–368.

Durmuller, N., Guillaume, P., Lacroix, P., Porsolt, R.D., Moser, P., 2007. The use of the dog electroencephalogram (EEG) in safety pharmacology to evaluate proconvulsant risk. J. Pharmacol. Toxicol. Methods 56, 234–238.

Easter, A., Sharp, T.H., Valentin, J.P., Pollard, C.E., 2007. Pharmacological validation of a semi-automated in vitro hippocampal brain slice assay for assessment of seizure liability. J. Pharmacol. Toxicol. Methods 56, 223–233.

EMA, 2006. Guideline on the non-clinical investigation of the dependence potential of medicinal products. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003360.pdf (pp.).

EMA, 2013. Guidance for Industry: Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf (pp.).

Emeigh Hart, S.G., 2005. Assessment of renal injury in vivo. J. Pharmacol. Toxicol. Methods 52, 30–45.

FDA, 2001. ICH S7A Safety Pharmacology Studies for Human Pharmaceuticals. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm074959.pdf (pp.).

FDA, 2005. S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals. http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm129122.pdf (pp.).

FDA, 2010. Guidance for Industry: Assessment of Abuse Potential of Drugs. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM198650.pdf.

Froger-Colleaux, C., Rompion, S., Guillaume, P., Porsolt, R.D., Castagne, V., Moser, P., 2011. Continuous evaluation of drug withdrawal in the rat using telemetry: effects of morphine and chlordiazepoxide. J. Pharmacol. Toxicol. Methods 64, 81–88.

Fuchs, T.C., Hewitt, P., 2011. Biomarkers for drug-induced renal damage and nephrotoxicity—an overview for applied toxicology. AAPS J. 13, 615–631.

Fung, M., T., Anna, Mybeck, Kathy, Hsiao-Hui, Jasmanda, Hornbuckle, Ken, Muniz, Edmundo, 2001. Evaluation of the characteristics of safety withdrawal of prescription drugs from worldwide pharmaceutical markets — 1960 to 1990. Drug Inf. J. 35, 293–317. Q20

Gacsalyi, U., Zabielski, R., Pierzynowski, S.G., 2000. Telemetry facilitates long-term recording of gastrointestinal myoelectrical activity in pigs. Exp. Physiol. 85, 239–241.

Gauvin, D.V., Yoder, J.D., Dalton, J.A., Baird, T.J., 2010. Comparisons of 3 plethysmography techniques for rodent pulmonary function assessment using ponemah waveforms analysis. J. Pharmacol. Toxicol. Methods 62, e32–e33.

Glennon, R.A., 1999. Arylalkylamine drugs of abuse: an overview of drug discrimination studies. Pharmacol. Biochem. Behav. 64, 251–256.

Globig, S., Witte, K., Lemmer, B., 1999. Urinary excretion of nitric oxide, cyclic GMP, and catecholamines during rest and activity period in transgenic hypertensive rats. Chronobiol. Int. 16, 305–314.

Goineau, S., Rompion, S., Guillaume, P., Picard, S., 2010. Ventilatory function assessment in safety pharmacology: optimization of rodent studies using normocapnic or hypercapnic conditions. Toxicol. Appl. Pharmacol. 247, 191–197.

Grant, A.O., 2009. Cardiac ion channels. Circ. Arrhythm. Electrophysiol. 2, 185–194.

Guth, B.D., Rast, G., 2010. Dealing with hERG liabilities early: diverse approaches to an important goal in drug development. Br. J. Pharmacol. 159, 22–24.

Guth, B.D., Bass, A.S., Briscoe, R., Chivers, S., Markert, M., Siegl, P.K., Valentin, J.P., 2009. Comparison of electrocardiographic analysis for risk of QT interval prolongation using safety pharmacology and toxicological studies. J. Pharmacol. Toxicol. Methods 60, 107–116.

Hancox, J.C., McPate, M.J., El Harchi, A., Zhang, Y.H., 2008. The hERG potassium channel and hERG screening for drug-induced torsades de pointes. Pharmacol. Ther. 119, 118–132.

Harrison, A.P., Erlwanger, K.H., Elbrond, V.S., Andersen, N.K., Unmack, M.A., 2004. Gastrointestinal-tract models and techniques for use in safety pharmacology. J. Pharmacol. Toxicol. Methods 49, 187–199.

Hayashi, E., Kobayashi, T., Shiroshita, Y., Kuratani, K., Kinoshita, M., Hara, H., 2011. An automated evaluation system for analyzing antinociceptive effects on intracolonic capsaicin-induced visceral pain-related licking behavior in mice. J. Pharmacol. Toxicol. Methods 64, 119–123.

Hernandez, S.H., Nelson, L.S., 2010. Prescription drug abuse: insight into the epidemic. Clin. Pharmacol. Ther. 88, 307–317.

Hollo, Z., Homolya, L., Davis, C.W., Sarkadi, B., 1994. Calcein accumulation as a fluorometric functional assay of the multidrug transporter. Biochim. Biophys. Acta 1191, 384–388.

Hoymann, H.G., 2007. Invasive and noninvasive lung function measurements in rodents. J. Pharmacol. Toxicol. Methods 55, 16–26.

Hoymann, H.G., 2012. Lung function measurements in rodents in safety pharmacology studies. Front. Pharmacol. 3, 156.

Irwin, S., 1968. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacologia 13, 222–257.

John-Baptiste, A., Huang, W., Kindt, E., Wu, A., Vitsky, A., Scott, W., Gross, C., Yang, A.H., Schaiff, W.T., Ramaiah, S.K., 2012. Evaluation of potential gastrointestinal biomarkers in a PAK4 inhibitor-treated preclinical toxicity model to address unmonitorable gastrointestinal toxicity. Toxicol. Pathol. Q21

Kaczorowski, G.J., Garcia, M.L., Bode, J., Hess, S.D., Patel, U.A., 2011. The importance of being profiled: improving drug candidate safety and efficacy using ion channel profiling. Front. Pharmacol. 2, 78.

Kagstrom, J., Sjogren, E.L., Ericson, A.C., 2007. Evaluation of the guinea pig monophasic action potential (MAP) assay in predicting drug-induced delay of ventricular repolarisation using 12 clinically documented drugs. J. Pharmacol. Toxicol. Methods 56, 186–193.

Kamendi, H.W., Brott, D.A., Chen, Y., Litwin, D.C., Lengel, D.J., Fonck, C., Bui, K.H., Gorko, M.A., Bialecki, R.A., 2010. Combining radio telemetry and automated blood sampling: a novel approach for integrative pharmacology and toxicology studies. J. Pharmacol. Toxicol. Methods 62, 30–39.

Kearney, K., Metea, M., Gleason, T., Edwards, T., Atterson, P., 2010. Evaluation of respiratory function in freely moving Beagle dogs using implanted impedance technology. J. Pharmacol. Toxicol. Methods 62, 119–126.

Kramer, K., Kinter, L.B., 2003. Evaluation and applications of radiotelemetry in small laboratory animals. Physiol. Genomics 13, 197–205.

Kramer, J.A., Sagartz, J.E., Morris, D.L., 2007. The application of discovery toxicology and pathology towards the design of safer pharmaceutical lead candidates. Nat. Rev. Drug Discov. 6, 636–649.

Kraushaar, U., Meyer, T., Hess, D., Gepstein, L., Mummery, C.L., Braam, S.R., Guenther, E., 2012. Cardiac safety pharmacology: from human ether-a-gogo related gene channel block towards induced pluripotent stem cell based disease models. Expert Opin. Drug Saf. 11, 285–298.

Laverty, H., Benson, C., Cartwright, E., Cross, M., Garland, C., Hammond, T., Holloway, C., McMahon, N., Milligan, J., Park, B., Pirmohamed, M., Pollard, C., Radford, J., Roome, N., Sager, P., Singh, S., Suter, T., Suter, W., Trafford, A., Volders, P., Wallis, R., Weaver, R., York, M., Valentin, J., 2011. How can we improve our understanding of cardiovascular safety liabilities to develop safer medicines? Br. J. Pharmacol. 163, 675–693.

Lawrence, C.L., Bridgland-Taylor, M.H., Pollard, C.E., Hammond, T.G., Valentin, J.P., 2006. A rabbit Langendorff heart proarrhythmia model: predictive value for clinical identification of Torsades de Pointes. Br. J. Pharmacol. 149, 845–860.

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Lemmer, B., Mattes, A., Bohm, M., Ganten, D., 1993. Circadian blood pressure variation in transgenic hypertensive rats. Hypertension 22, 97–101.

Levi, F., Hrushesky, W.J., Borch, R.F., Pleasants, M.E., Kennedy, B.J., Halberg, F., 1982. Cisplatin urinary pharmacokinetics and nephrotoxicity: a common circadian mechanism. Cancer Treat. Rep. 66, 1933–1938.

Lienemann, K., Plotz, T., Pestel, S., 2008. NMR-based urine analysis in rats: prediction of proximal tubule kidney toxicity and phospholipidosis. J. Pharmacol. Toxicol. Methods 58, 41–49.

Lindgren, S., Bass, A.S., Briscoe, R., Bruse, K., Friedrichs, G.S., Kallman, M.J., Markgraf, C., Patmore, L., Pugsley, M.K., 2008. Benchmarking safety pharmacology regulatory packages and best practice. J. Pharmacol. Toxicol. Methods 58, 99–109.

Litwin, D.C., Lengel, D.J., Kamendi, H.W., Bialecki, R.A., 2011. An integrative pharmacological approach to radio telemetry and blood sampling in pharmaceutical drug discovery and safety assessment. Biomed. Eng. Online 10, 5.

Luft, J., Bode, G., 2002. Integration of safety pharmacology endpoints into toxicology studies. Fundam. Clin. Pharmacol. 16, 91–103.

Lynch III, J.J., Castagne, V., Moser, P.C., Mittelstadt, S.W., 2011. Comparison of methods for the assessment of locomotor activity in rodent safety pharmacology studies. J. Pharmacol. Toxicol. Methods 64, 74–80.

Malik, M., Farbom, P., Batchvarov, V., Hnatkova, K., Camm, A.J., 2002. Relation between QT and RR intervals is highly individual among healthy subjects: implications for heart rate correction of the QT interval. Heart 87, 220–228.

McGrath, P., Li, C.Q., 2008. Zebrafish: a predictive model for assessing drug-induced toxicity. Drug Discov. Today 13, 394–401.

McMahon, C., Mitchell, A.Z., Klein, J.L., Jenkins, A.C., Sarazan, R.D., 2010. Evaluation of blood pressure measurement using a miniature blood pressure transmitter with jacketed external telemetry in cynomolgus monkeys. J. Pharmacol. Toxicol. Methods 62, 127–135.

Meyer, O., Jenni, R., Greiter-Wilke, A., Breidenbach, A., Holzgrefe, H.H., 2010. Comparison of telemetry and high-definition oscillometry for blood pressure measurements in conscious dogs: effects of torcetrapib. J. Am. Assoc. Lab. Anim. Sci. 49, 464–471.

Miller, D.S., 2002. Xenobiotic export pumps, endothelin signaling, and tubular nephrotoxicants—a case of molecular hijacking. J. Biochem. Mol. Toxicol. 16, 121–127.

Monahan, B.P., Ferguson, C.L., Killeavy, E.S., Lloyd, B.K., Troy, J., Cantilena Jr., L.R., 1990. Torsades de pointes occurring in association with terfenadine use. JAMA 264, 2788–2790.

Moretti, A., Bellin, M., Welling, A., Jung, C.B., Lam, J.T., Bott-Flugel, L., Dorn, T., Goedel, A., Hohnke, C., Hofmann, F., Seyfarth, M., Sinnecker, D., Schomig, A., Laugwitz, K.L., 2010. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. N. Engl. J. Med. 363, 1397–1409.

Moscardo, E., McPhie, G., Fasdelli, N., Giarola, A., Tontodonati, M., Dorigatti, R., Meecham, K., 2010. An integrated cardiovascular and neurobehavioural functional assessment in the conscious telemetered cynomolgus monkey. J. Pharmacol. Toxicol. Methods 62, 95–106.

Moser, P., Wolinsky, T., Castagne, V., Duxon, M., 2011. Current approaches and issues in non-clinical evaluation of abuse and dependence. J. Pharmacol. Toxicol. Methods 63, 160–167.

Muller, P.Y., Dieterle, F., 2009. Tissue-specific, non-invasive toxicity biomarkers: translation from preclinical safety assessment to clinical safety monitoring. Expert Opin. Drug Metab. Toxicol. 5, 1023–1038.

Murphy, D.J., 2002. Assessment of respiratory function in safety pharmacology. Fundam. Clin. Pharmacol. 16, 183–196.

Murphy, D.J., Renninger, J.P., Schramek, D., 2010. Respiratory inductive plethysmography as a method for measuring ventilatory parameters in conscious, non-restrained dogs. J. Pharmacol. Toxicol. Methods 62, 47–53.

Nattel, S., Carlsson, L., 2006. Innovative approaches to anti-arrhythmic drug therapy. Nat. Rev. Drug Discov. 5, 1034–1049.

Ninkovic, J., Bally-Cuif, L., 2006. The zebrafish as a model system for assessing the reinforcing properties of drugs of abuse. Methods 39, 262–274.

Nirogi, R., Shanmuganathan, D., Jayarajan, P., Abraham, R., Kancharla, B., 2012. Comparison of whole body and head out plethysmography using respiratory stimulant and depressant in conscious rats. J. Pharmacol. Toxicol. Methods 65, 37–43.

Peng, S., Lacerda, A.E., Kirsch, G.E., Brown, A.M., Bruening-Wright, A., 2010. The action potential and comparative pharmacology of stem cell-derived human cardiomyocytes. J. Pharmacol. Toxicol. Methods 61, 277–286.

Pestel, S., Martin, H.J., Maier, G.M., Guth, B., 2006. Effect of commonly used vehicles on gastrointestinal, renal, and liver function in rats. J. Pharmacol. Toxicol. Methods 54, 200–214.

Pestel, S., Krzykalla, V., Weckesser, G., 2007. Measurement of glomerular filtration rate in the conscious rat. J. Pharmacol. Toxicol. Methods 56, 277–289.

Pirmohamed, M., James, S., Meakin, S., Green, C., Scott, A.K., Walley, T.J., Farrar, K., Park, B.K., Breckenridge, A.M., 2004. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. BMJ 329, 15–19.

Pons, M., Schnecko, A., Witte, K., Lemmer, B., Waterhouse, J.M., Cambar, J., 1996. Circadian rhythms in renal function in hypertensive TGR(mRen-2)27 rats and their normotensive controls. Am. J. Physiol. 271, R1002–R1008.

Porsolt, R.D., Lemaire, M., Durmuller, N., Roux, S., 2002. New perspectives in CNS safety pharmacology. Fundam. Clin. Pharmacol. 16, 197–207.

Porsolt, R.D., C.D., Niklaus, Lemaire, Martine, Moser, Paul, Rous, Slyvain, France, Charles P., 2006. Central Nervous System (CNS) Safety Pharmacology Studies. Springer, Germany. Q22

Pugsley, M.K., Authier, S., Curtis, M.J., 2008. Principles of safety pharmacology. Br. J. Pharmacol. 154, 1382–1399.

Rahma, M., Kimura, S., Yoneyama, H., Kosaka, H., Nishiyama, A., Fukui, T., Abe, Y., 2001. Effects of furosemide on the tubular reabsorption of nitrates in anesthetized dogs. Eur. J. Pharmacol. 428, 113–119.

Redfern, W.S., Carlsson, L., Davis, A.S., Lynch, W.G., MacKenzie, I., Palethorpe, S., Siegl, P.K., Strang, I., Sullivan, A.T., Wallis, R., Camm, A.J., Hammond, T.G., 2003. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovasc. Res. 58, 32–45.

Redfern, W.S., Strang, I., Storey, S., Heys, C., Barnard, C., Lawton, K., Hammond, T.G., Valentin, J.P., 2005. Spectrum of effects detected in the rat functional observational battery following oral administration of non-CNS targeted compounds. J. Pharmacol. Toxicol. Methods 52, 77–82.

Redfern, W.S., Waldron, G., Winter, M.J., Butler, P., Holbrook, M., Wallis, R., Valentin, J.P., 2008. Zebrafish assays as early safety pharmacology screens: paradigm shift or red herring? J. Pharmacol. Toxicol. Methods 58, 110–117.

Samson, N., Dumont, S., Specq, M.L., Praud, J.P., 2011. Radio telemetry devices to monitor breathing in non-sedated animals. Respir. Physiol. Neurobiol. 179, 111–118.

Shah, R.R., 2006. Can pharmacogenetics help rescue drugs withdrawn from the market? Pharmacogenomics 7, 889–908.

Shell, L., Rozum, M., Jortner, B.S., Ehrich, M., 1992. Neurotoxicity of acrylamide and 2,5-hexanedione in rats evaluated using a functional observational battery and pathological examination. Neurotoxicol. Teratol. 14, 273–283.

Stonard, M.D., 1990. Assessment of renal function and damage in animal species. A review of the current approach of the academic, governmental and industrial institutions represented by the Animal Clinical Chemistry Association. J. Appl. Toxicol. 10, 267–274.

Talavera, J., Kirschvink, N., Schuller, S., Garreres, A.L., Gustin, P., Detilleux, J., Clercx, C., 2006. Evaluation of respiratory function by barometric whole-body plethysmography in healthy dogs. Vet. J. 172, 67–77.

Tontodonati, M., Fasdelli, N., Moscardo, E., Giarola, A., Dorigatti, R., 2007. A canine model used to simultaneously assess potential neurobehavioural and cardiovascular effects of candidate drugs. J. Pharmacol. Toxicol. Methods 56, 265–275.

Trepakova, E.S., Koerner, J., Pettit, S.D., Valentin, J.P., Committee, H.P.-A., 2009. A HESI consortium approach to assess the human predictive value of non-clinical repolarization assays. J. Pharmacol. Toxicol. Methods 60, 45–50.

Valentin, J.P., Hammond, T., 2008. Safety and secondary pharmacology: successes, threats, challenges and opportunities. J. Pharmacol. Toxicol. Methods 58, 77–87.

Valentin, J.P., Bass, A.S., Atrakchi, A., Olejniczak, K., Kannosuke, F., 2005. Challenges and lessons learned since implementation of the safety pharmacology guidance ICH S7A. J. Pharmacol. Toxicol. Methods 52, 22–29.

Valentin, J.P., Bialecki, R., Ewart, L., Hammond, T., Leishmann, D., Lindgren, S., Martinez, V., Pollard, C., Redfern, W., Wallis, R., 2009. A framework to assess the translation of safety pharmacology data to humans. J. Pharmacol. Toxicol. Methods 60, 152–158.

Wallis, R.M., 2010. Integrated risk assessment and predictive value to humans of non-clinical repolarization assays. Br. J. Pharmacol. 159, 115–121.

West, R., Gossop, M., 1994. Overview: a comparison of withdrawal symptoms from different drug classes. Addiction 89, 1483–1489.

Wienkers, L.C., Heath, T.G., 2005. Predicting in vivo drug interactions from in vitro drug discovery data. Nat. Rev. Drug Discov. 4, 825–833.

Winter, M.J., Redfern, W.S., Hayfield, A.J., Owen, S.F., Valentin, J.P., Hutchinson, T.H., 2008. Validation of a larval zebrafish locomotor assay for assessing the seizure liability of early-stage development drugs. J. Pharmacol. Toxicol. Methods 57, 176–187.

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