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The Altascientist

SCIENTIFIC JOURNAL

ISSUE NO. 13

SAFETY PHARMACOLOGY GUIDELINES AND PRACTICES – A REVIEW

Safety pharmacology studies are defined in the International Conference on Harmonization (ICH) S7A guidance[1] as "those studies that investigate the potential undesirable pharmacodynamic effects of a substance on physiological functions in relation to exposure in the therapeutic range and above." In other words, they are a set of tests focused on potentially adverse effects of pharmaceutical or biological agents, differing from traditional toxicology studies as they are typically concentrated on a single organ or physiological system.

Safety pharmacology studies should have a clearly identified and delineated investigational plan to:

1. Identify undesirable pharmacodynamic properties of a substance that may have relevance to its human safety.
2. Evaluate adverse pharmacodynamic and/or pathophysiological effects of a substance observed in toxicology and/or clinical studies.
3. Investigate the mechanism of the adverse pharmacodynamic effects observed and/or suspected.

Bearing in mind the objectives and the unique properties of the test article, there are a number of points to consider in selecting and designing a program of safety pharmacology studies:

1. Effects directly related to the test substance's therapeutic class — In the S7A guideline, the example of proarrhythmia caused by antiarrhythmic agents is provided as an illustration of a mechanism of action causing specific adverse effects.
2. Adverse effects associated with the chemical or therapeutic class, but independent of the primary pharmacodynamic effects — The classic example given by S7A is anti-psychotics and QT prolongation, which in fact is the subject of a completely separate guideline.
3. Ligand binding or enzyme assays which may suggest potential for adverse effects.
4. Results from previous studies (safety pharmacology, pharmacodynamic, or toxicology), or from human use that would warrant further investigation.

There is a core battery of safety pharmacology studies designed to investigate the effects of the test substance on vital functions. The vital organ systems typically considered are the cardiovascular, respiratory, and central nervous systems (CNS); these should be included in the core battery, and studies should be conducted before human exposure, in accordance with ICH S7A and S7B.[2] In some instances, based on scientific rationale, the core battery need not be implemented or should be supplemented.

The core battery meets worldwide regulatory requirements, and is the backbone of the vast majority of safety pharmacology programs. Combination of the cardiovascular and respiratory assessments in the selected non-rodent species is a suitable approach that can have beneficial impacts on cost and resources.

Safety pharmacology studies are required to be conducted in accordance with Good Laboratory Practices (GLP). It is recognized that due to their unique design, it may not be possible to conduct all studies in a GLP-compliant manner. However, all reasonable attempts should be made to perform them within the framework of GLP.

Other organ systems, such as the renal or gastrointestinal (GI), which may be affected in a transient manner without causing irreversible damage or death, are not included in the core battery, and would generally be examined in supplementary studies. That said, these non-core systems may take on greater importance in specific human clinical patient populations; for example, in the assessment of effects on the GI tract for Crohn's disease and on renal function in primary renal hypertension.

Considerable information concerning the properties and behavior of a test article is necessary before embarking on a safety pharmacology program. This impacts the timing of studies with respect to clinical development. As a minimum, the core battery should be performed prior to first administration in humans, along with any supplemental studies to address specific causes for concern.

CORE AND SUPPLEMENTAL STUDIES

The following list is not meant to be comprehensive or prescriptive, and as discussed earlier, the studies should be selected on a case-by-case basis for a particular test article. In some cases, it may be appropriate to address certain effects during the conduct of other nonclinical and/or clinical studies.

Table 1. Core Studies

CORE BATTERY		
Study Type	**Species**	**Follow-Up Studies**
Cardiovascular Blood pressure, heart rate, and the electrocardiogram should be evaluated. *In vivo, in vitro* and/or *ex vivo* evaluations, including methods for repolarization and conductance abnormalities, should also be considered.	Same as non-rodent* species in toxicology studies	Cardiac output, ventricular contractility, vascular resistance, the effects of endogenous and/or exogenous substances on the cardiovascular responses
Central Nervous System Motor activity, behavioral changes, coordination, sensory/motor reflex responses and body temperature should be evaluated. For example, a functional observation battery (FOB), modified Irwin's, or other appropriate test can be used.	Generally, the same rodent species used in toxicology studies	Behavioral pharmacology, learning and memory, ligand-specific binding, neurochemistry, visual, auditory, and/or electrophysiology examinations
Respiratory Respiratory rate and other measures of respiratory function (e.g., tidal volume or hemoglobin oxygen saturation) should be evaluated.	Same as non-rodent* or rodent species in toxicology studies	Airway resistance, compliance, pulmonary arterial pressure, blood gases, blood pH, etc.
**Respiratory assessments can be combined with cardiovascular evaluations in the non-rodent species using surgically implanted telemetry.*		

Table 2. Supplemental Studies

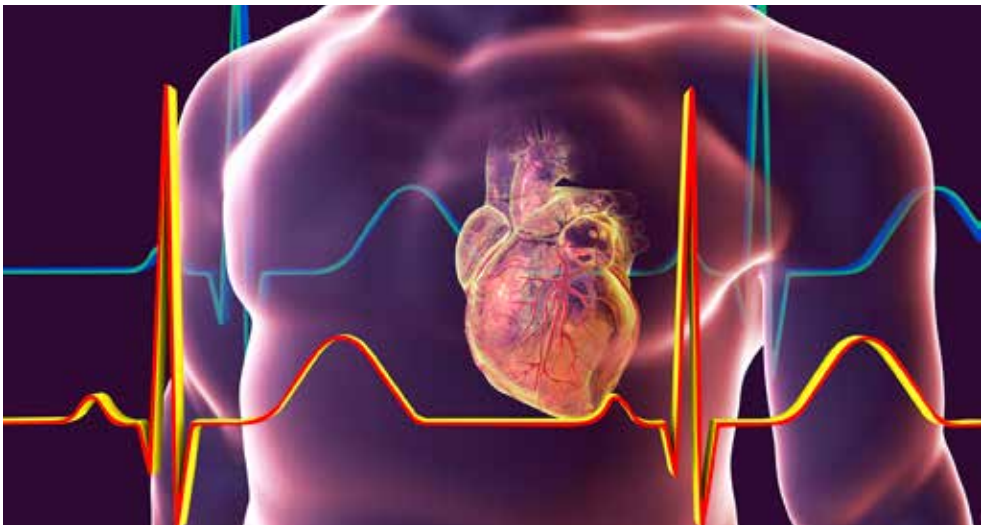
SUPPLEMENTAL STUDIES (Implementation based on scientific rationale)
Renal/Urinary System Urinary volume, specific gravity, osmolality, pH, fluid/electrolyte balance, proteins, cytology, and blood chemistry determinations, such as blood urea nitrogen, creatinine, and plasma proteins can be used.
Autonomic Nervous System Binding to receptors relevant for the autonomic nervous system, functional responses to agonists or antagonists *in vivo* or *in vitro*, direct stimulation of autonomic nerves and measurement of cardiovascular responses, baroreflex testing, and heart rate variability can be used.
Gastrointestinal System Gastric secretion, gastrointestinal injury potential, bile secretion, transit time *in vivo*, ileal contraction *in vitro*, gastric pH measurement, and pooling can be used.
Other Organ Systems Effects of the test substance on organ systems not investigated elsewhere should be assessed when there is a reason for concern. For example, dependency potential or skeletal muscle, immune, and endocrine functions can be investigated.

Cardiovascular System

The endpoints for the cardiovascular system in the S7A guideline include blood pressure, heart rate, and electrocardiogram (ECG) evaluation. Standard measurements for blood pressure include systolic and diastolic readings. Quantitative and qualitative cardiology evaluations are performed from lead II, and the heart rate is typically derived from the RR interval on the ECG. Measurement of the QT interval is necessary to comply with the ICH S7B guidance.

It is suggested to perform *in vivo* and *ex vivo/ in vitro* evaluations, including repolarization and conductance abnormalities. *In vitro* studies, for example hERG, will permit the evaluation of repolarization of the cardiac human cells when exposed to the drug. *In vivo* studies are typically performed on freely moving, conscious, telemetered dogs or primates. An anesthetized study is acceptable if toxicity or lack of exposure prevent the use of freely moving animals.

Although the S7A guidance recommends telemetry as first choice because it allows animals to be unrestrained and conscious, thus in a "normal" environment, the data collected using commercially available systems are limited. The core battery requirement can be assessed. However, for some test articles, it may be appropriate to consider performing a complete cardiovascular profile, including assessment of pulmonary artery pressure, cardiac output, stroke volume, left ventricular pressure, and rates of ventricular contraction and relaxation. By collecting these data early, possible need for follow-up studies can be reduced and potential development delays can be avoided. The main drawback to this approach is the relatively short duration (days) when compared to telemetry (months), and the increased potential for artifacts related to changes in the position of catheters used for measurements. The investigator has a choice to make when considering the cardiovascular studies, and the specific properties of the test article need to be carefully considered before a decision is made.



Central Nervous System

The CNS endpoints include motor activity, behavioral changes, coordination, sensory/motor reflex responses, and body temperature.

S7A describes a number of evaluations which should be performed, the majority of which are included in a standard FOB or modified Irwin Screen.

The FOB and modified Irwin Screen have a wealth of historical data regarding their performance and use in evaluation of numerous test articles of different classes. However, due to the subjective nature of the evaluation and interpretation of some behavioral responses, these assessments should be performed by experienced, well-trained personnel[3], and their training should involve the use of positive control articles, which will cause a wide range of changes in behavior.[4]

Study Sequence — Modified Irwin's Screen

Figure 1.

HOME OBSERVATIONS	OPEN FIELD ACTIVITY	AUTONOMIC ACTIVITY ASSESSMENT (SENSORIMOTOR REFLEX)
CAGE: Posture and unusual behaviors (possible convulsion, shivering, vocalization, stimulation, stereotypy)	Spontaneous motor activity, rears, gait abnormalities, stereotypic behavior, irritability, body tone, salivation, lacrimation, piloerection, catalepsy, micturition, defecation	Touch response (passivity), startle response, righting reflex, palpebral reflex, tail pinch, grasping reflex, ataxia, rectal temperature, pupil response

Respiratory System

Respiratory rate and other measures of respiratory function (e.g., tidal volume and/or hemoglobin O_2 saturation) should be evaluated, using appropriate methodologies. Whole-body plethysmography in freely moving, conscious rats is one of the standard approaches. Clinical observations of animals are generally not sufficient to assess respiratory function.

Combination of the cardiovascular and respiratory assessments in the selected non-rodent species is also a suitable approach, using surgically implanted animals. This combination permits study of time-dependent effects, and allows for insight into possible mechanisms of action. In addition, since two of the three core assessments can be combined, this approach can have beneficial impacts on decreasing the number of animals used (3Rs), cost, and other resources.

Species Selection

There are a number of test system options that can be explored. Historical models of safety evaluation drive the design for the majority of studies, i.e., rats used for CNS and respiratory studies, dogs for cardiovascular. More recently, and with the appropriate technology, respiratory assessments can be combined with cardiovascular studies in dogs and monkeys.

Sometimes, traditional models may not be the most appropriate for the test article and, in those cases, consideration should be given to pharmacological responsiveness, pharmacokinetic profile, sensitivity, and available background data. When departing from the standard test systems, it is important that the use of the animal model can be justified based on evaluation of the above factors. As discussed previously, the guideline states that data from conscious, unrestrained, unanesthetized animals is preferred, and that the use of chronically instrumented (telemetered) animals should be seriously considered.

Number of Animals

Group size for each assay is another important consideration in study design. S7A does not define a specific requirement, and states that the group sizes should be sufficient to allow meaningful interpretation of data and demonstrate the presence or absence of a biologically significant effect of the test article. For CNS and respiratory studies in the core battery, power analysis of raw data has shown that group sizes of six and eight males, respectively, will meet and generally exceed the desired power of 80%.[5] For cardiovascular studies, various designs can be employed, possibly involving increasing doses of the test article following a washout period, or variations of crossover designs. Thus, the number of animals will vary from study to study.

Route of Administration

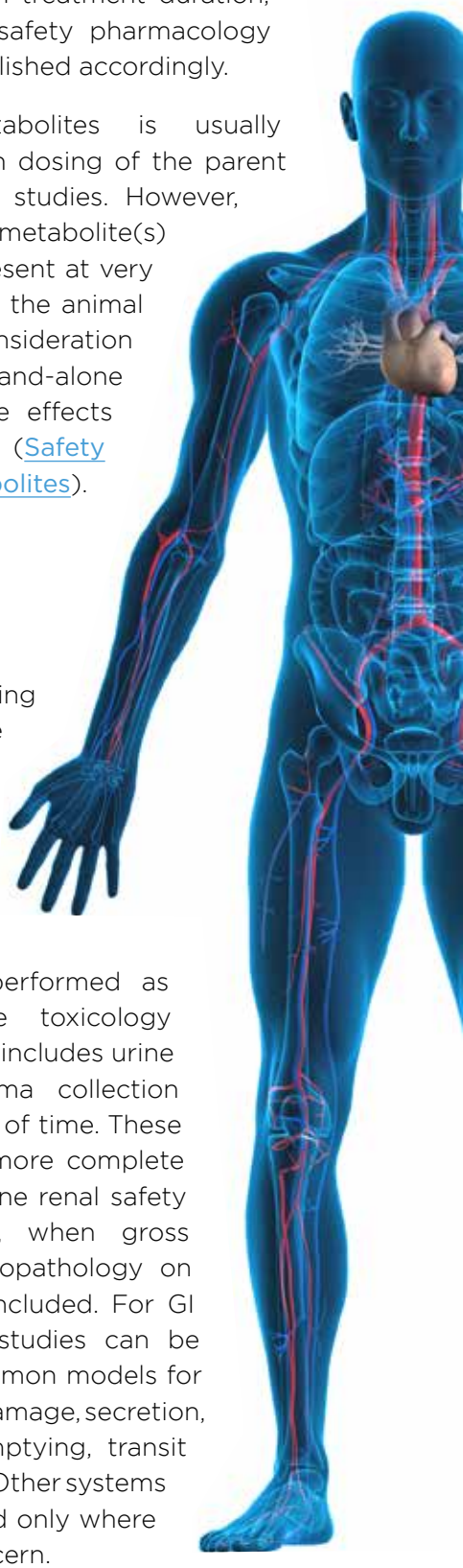
The route of administration for safety pharmacology studies should generally be the same as the intended clinical route whenever possible. When the expected clinical route is not possible, it is important to ensure that exposure to the parent compound and major metabolites is similar to, or greater than, that achieved in humans.

Dose Level Selection and Duration

Safety pharmacology studies should define a dose-response relationship for any observed adverse effects, with evaluation of the onset and duration of the response. Using core battery studies as an example, assessments are typically performed around the time of C_{max} and during the elimination phase to show potential for delayed onset or reversibility of any effects. Dose levels should include and exceed the therapeutic range. In the absence of an adverse effect on the parameter being evaluated, the maximum dose should be one that produces moderate effects in studies of a similar route and duration.

For practical reasons, toxic effects at these dose levels (i.e., tremors during ECG recording) may confound interpretation of results and serve to limit dose levels used in the study. The studies should usually be performed as single-dose administrations. In cases where certain responses may present only following a given treatment duration, the duration of the safety pharmacology study should be established accordingly.

Evaluation of metabolites is usually accomplished through dosing of the parent compound in animal studies. However, the major human metabolite(s) may be absent, or present at very low concentrations, in the animal model. As such, consideration should be given to stand-alone studies to assess the effects of these metabolites (Safety Testing of Drug Metabolites).



Supplemental (Follow-Up) Studies

Follow-up studies as described in Table 2 may be required based on the pharmacological properties of the test article, or if concerns arise from the core battery studies, clinical trials, pharmacovigilance, or other sources. For supplemental CNS testing, there are a number of models to choose from, depending on the pharmacodynamics of the drug. Some that are considered most relevant include evaluation of the potential for pro-convulsant activity, assessments of auditory or visual changes, potential drug interaction or analgesic activity, and effects on learning and memory. Supplemental cardiovascular evaluation (as discussed above in comparing methods for the core battery) can all be collected in one study if the decision is made to perform a full, early stage cardiovascular profile. Otherwise, the additional data is not usually challenging to obtain via a standard animal model.

Supplemental evaluation of respiratory parameters can present a challenge, as models available to assess the required effects on airway resistance and compliance may involve the use of anesthetized animals. Alternative designs[6] are available. However, these are only required if the core battery assessment (or other sources) raise concerns.

Among the remaining systems, those commonly tested in supplemental studies are the renal and GI systems. Renal safety pharmacology evaluation can be performed as part of single-dose toxicology studies, if their design includes urine and serum or plasma collection over a suitable period of time. These studies can provide more complete data than a stand-alone renal safety pharmacology study, when gross examination and histopathology on relevant tissues are included. For GI assessment, various studies can be conducted, using common models for evaluation of gastric damage, secretion, pH measurement, emptying, transit time, and contraction. Other systems should be investigated only where there is cause for concern.

SAFETY PHARMACOLOGY ENDPOINTS ON TRADITIONAL TOXICOLOGY STUDIES

Safety pharmacology endpoints can be included in the regulatory single- or repeat-dose toxicology studies. In those instances, species selection is important, as administration by certain routes in certain species may not attain the level of exposure required.

In some cases, combination studies, where integrated approaches examine the inter-relationships of drug-mediated effects on different organs, can be a practical approach for safety pharmacology studies. One example is the addition of FOB/Irwin's Screen to rodent toxicology studies. The additional assessments are usually performed on a subset of animals, typically from the population of animals assigned to the evaluation of potential toxicity (generally between three to five animals/sex/group).

The addition of cardiovascular endpoints is also common on non-rodent toxicology studies. These would be captured typically via jacketed external telemetry (JET). Blood pressure can also be monitored via an implant or indirectly. Arterial blood gas for hemoglobin O_2 saturation can also be added to assess the respiratory function in non-rodents.

Integration of safety pharmacology endpoints in general toxicity studies can reduce the total number of individual studies and total number of animals used, and aid in integrative interpretations. Combination studies also have 3R benefits, and can have a beneficial impact on cost and resources.

When designing integrated studies, it is important to consider that safety pharmacology requirements must not confound interpretation of general toxicology endpoints, and vice versa. As examples, surgery for implants may introduce pathological artifacts while the extra handling and/or novel environments can induce stress artifacts.

Safety Pharmacology Studies May Not Be Necessary

According to section 2.9 of the ICH S7B guidance, safety pharmacology studies may not be necessary in cases of locally applied agents (dermal or ocular), where the pharmacology is well characterized, and systemic exposure or distribution to other organs is demonstrated to be low. The same applies for cytotoxic agents for treatment of end-stage cancer (for agents with novel mechanisms of action, safety pharmacology studies may be of value), biotechnology-derived products, and new formulations having similar PK and PD properties.

Choosing a CRO for Safety Pharmacology Studies

Safety pharmacology may or may not be required to move your molecule forward. Altasciences' experts can provide insight for the fundamental considerations in selecting and designing a suitable program of safety pharmacology. Establishing a specific roadmap which includes the core battery (cardiovascular, respiratory, and central nervous systems) is the starting point, with supplemental studies included when there is a cause for concern. Some safety pharmacology endpoints can be included in the regulatory single- or repeat-dose toxicology studies; it is a wise decision to consult our scientists for a thorough review of your program requirements, and how we can assist you with a seamless transition to regulatory submission, and human clinical trials.

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