Drug Safety Evaluation: Methods and Protocols

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Drug discovery and development is a long and expensive process. It is estimated to take an average of 10–15 years and up to $800 million to develop a new drug. Furthermore, on average only five out of 250 compounds selected from *in vitro* drug discovery programs for pre-clinical testing will enter clinical trials. In the majority of cases this is because either the compound did not show sufficient therapeutic activity *in vivo* or it had adverse effects and was therefore considered unsafe.

Non-clinical safety assessment is an important component of the overall drug development plans for new drug candidates (leads) and biologics. Laboratory studies including *in vitro* and in animals systems, are an essential step in the progress of new pharmaceuticals heading toward the ultimate goal of clinical trials and, eventually, approval.

In the book Drug Safety Evaluation: Methods and Protocols, expert researchers detail a compendium of analytical technologies with a focus on applicability in real life laboratory practice. As a volume in the successful Methods in Molecular Biology™ series, chapters include brief introductions to their respective subjects, lists of the necessary materials, step-by-step guides through the techniques and protocols.

The book is divided into eight parts. Part I: General Toxicology; Part II: Pathology; Part III: Genetic Toxicology; Part IV: Safety Pharmacology; Part V: Generation and Analysis of Transcriptomics Data; Part VI: Screening Assays for Developmental Toxicity; Part VII: Chemical Protein Adducts; and Part VIII focuses on Safety Biomarkers.

In Part I the authors discuss the combination drugs in preclinical studies and the preclinical evaluation of juvenile toxicity. We know that these topics are of great interest to those working in the drug safety evaluation, because the combination of drugs has the potential for pharmacokinetic and/or toxicological interaction between the components; and a pediatric assessment is now a required component of every New Drug Application in North America or Marketing Authorization Application in Europe.

Part II comprises four chapters all related with Pathology. It is discussed necropsy and sampling procedures in rodents, histopathology procedures from tissue sampling to histopathological evaluation, principles and methods of immunohistochemistry and tissue microarrays and digital image analysis. I recommend the last chapter because tissue microarrays (TMAs) have recently emerged as very valuable tools for high-throughput pathological assessment, especially in cancer research arena. The authors describe the process of digital slide scanning of kidney and liver sections, in the context of creating an online resource of histopathological data.

Micronucleus assay and labeling of centromeres with FISH technique, and the use of bacterial repair endonucleases in the comet assay are techniques discussed in Part III. The comet assay (single cell gel electrophoresis) is widely used in genetic toxicology for measuring DNA damage in the form of strand breaks. It can be applied to cells from animal tissues after *in vivo* testing or to cultured cells used *in vitro* experiments.

In Part IV the authors have described manual whole-cell patch-clamping of the HERG cardiac K+ channel. It is an important technique to those working with drug evaluation, since a major safety concerning the drug development process is a phenomenon known as acquired long QT syndrome, which occurs when a drug, often as an unintended side effect, delays the repolarization process of the heart (1). Over the past decade, a number of marketed drugs have been withdrawn, or their use severely restricted, because they produced QT prolongation and *torsades de pointes* arrhythmia. These include drugs from many pharmacological classes, including antipsychotics (pimozide, sertindole), antihistamines (terfenadine, astemizole), antibiotics (grepafloxacin), and gastric prokinetics (cisapride) (2). For this reason, elimination of drug/HERG interactions has become a major focus of the pharmaceutical industry and has led to the creation of a branch of safety pharmacology that specifically studies HERG and the consequences of its inhibition.

The Investigative Toxicology, title of the Part V, comprises five chapters: Generation and analysis of transcriptomics data, Protocols of two-dimensional difference gel electrophoresis (2D-DIGE) to investigate mechanisms of toxicity, Protocols and applications of cellular metabolomics in safety studies using precision-cut tissue slices and carbon 13 NMR, Statistical

analysis of quantitative RT-PCR results, and Evaluation of mitochondrial respiration in cultured rat hepatocytes. Although all the chapters have suitable content to the description of their techniques, I highlight the chapter twelve, "Protocols and applications of cellular metabolomics in safety studies using precision-cut tissue slices and carbon 13 NMR", because the approach provides a panoramic view not only of all the pathways involved in the metabolism of ant given substrate in any human or animal cell type *in vitro*, but also of the beneficial and adverse effects of xenobiotics on these metabolic pathways.

The two chapters of the Part VI discuss FETAX assay for evaluation of developmental toxicity and Evaluation of embryotoxicity using the zebrafish model. Development toxicity represents an important issue for drug development. A teratogenic potential found prior to preclinical trials is a major benefit to initiate earlier appropriate *in vivo* studies to confirm or not the alert. However, in early development stage, the number of compounds to be tested is large and their available quantities are generally low. Regarding these requirements, Frog Embryo Teratogenesis Assay Xenopus (FETAX) is adapted to evaluate developmental toxicity potential using small drug quantites compared to *in vivo* studies. The embryonic zebrasih model offers the power of whole-animal investigations with the convenience of cell culture. Zebrafish (*Danio rerio*) offer a number of practical advantages as a model organism, making these vertebrates highly amenable for toxicologically relevant research. Is can be employed as a powerful *in vivo* model system to assess biological interactions and is an outstanding platform to detail the mechanisms by which substances elicit specific biological responses. Because zebrafish can be used at multiple stages in drug discovery, there is the potential to obtain *in vivo* data on both efficacy and safety at the earliest possible opportunity thereby potentially reducing attrition in the drug discovery process (3). Larval zebrafish assays have been utilized in the pharmaceutical industry in several different aspects of drug discovery namely target validation, toxicology and safety pharmacology assessment, disease modeling and drug reprofiling.

Chemical Protein Adducts, Part VII, discuss one of the most interesting and important issues of the book in my opinion. Xenobiotics can produce a variety of beneficial, as well as adverse effects in mammals. One potential source of drug-mediated toxicity stems from metabolic activation of the parent compound, typically catalyzed by one or more members of the cytochrome P450 family of enzymes. The resulting electrophile, if not quenched by endogenous nucleophiles, can form covalent adducts to cellular proteins, potentially resulting in enzyme inactivation, cell death, or formation of an immunogenic species. The toxicological consequences of exposure to such reactive intermediates range from mild inflammation to organ failure, anaphylaxis, and death. The five chapters of this Part discuss current techniques to deal with chemical protein adducts, including Protocols of i*n vitro* protein covalent binding studies in liver, Utilization of MALDI-TOF to determine chemical-protein adduct formation *in vitro*, Utilization of LC-MS/MS analyses to identify site-specific chemical protein adducts *in vitro*, One-dimensional western blotting coupled to LC-MS/MS analysis to identify chemical-adducted proteins in rat urine, and Identification of chemical adducted proteins in urine by multi-dimensional protein identification technology (LC/LC-MS/MS). Since drug-induced liver injury remains the single most important cause of FDA drug non-approvals and withdrawals (4), these five chapters are of great importance to evaluate drug safety in a preclinical setting.

At the end but not least, there is the four chapters of the Part VIII, which discuss Safety biomarkers: Optimization of SELDI for biomarker detection in plasma, Differential proteomics incorporating iTRAQ labelling and multi-dimensional separations, NMR and MS methods for metabonomics, and Absolute quantification of toxicological biomarkers by multiple reaction monitoring.

A table of contents, a listing of contributing authors, and a general index are included. Drug Safety Evaluation: Methods and Protocols serves as a guide to this field, helpful to medicinal chemists, toxicologists, biochemists, and molecular biologists as well as scientists from all other disciplines who wish to translate these methods into their own work.

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