
Preface

Non-clinical drug safety evaluation is the assessment of the safety profile of therapeutic agents through the conduct of laboratory studies in *in vitro* systems and in animals. The main objectives of drug safety evaluation studies are to differentiate between new drug entities that are unacceptably toxic and those that are not, characterize the potential adverse effects of new drugs, determine animal dosage levels that do not cause toxicity, and to estimate safe dosages to be used in clinical studies. Several types of studies are conducted in drug safety evaluation: acute to chronic general toxicity studies, reproductive toxicity studies, genotoxicity studies, carcinogenicity studies, safety pharmacology studies, and investigative toxicity studies.

General toxicity studies are usually performed in a rodent and in a nonrodent species to determine target organs of toxicity and evaluate doses of a new drug candidate that can be safely administered to man. In this book, specific aspects related to the experimental design of toxicity studies conducted to support drug combinations in humans and pediatric indications are described in the reviews of Chaps. 1 and 2, respectively. In general toxicity studies, the key traditional endpoints evaluated include clinical signs, clinical pathology parameters, along with macroscopic examination of organs at necropsy and light microscopic examination of a comprehensive list of tissues. Chapter 3 details the necropsy and sampling procedures used in rodents, and Chap. 4 highlights the histopathology procedures from tissue sampling to histopathological evaluation. Chapters 5 and 6 describe additional methods, such as immunohistochemistry, tissue microarrays, and digital image analysis, which can be used to complete and refine the traditional histopathological examination of organs.

Genotoxicity studies are carried out to evaluate the potential of new drug candidates to induce mutations and/or chromosomal damages. Chapter 7 presents the method of the micronucleus assay and its combination with centromeric labeling in the fluorescence *in situ* hybridization (FISH) technique to detect aneugenic events. Chapter 8 describes the comet assay, a sensitive electrophoretic method for measuring DNA strand breaks at the level of single cells, together with the use of bacterial repair endonucleases to detect specific DNA lesions.

Safety pharmacology studies are conducted to evaluate the effect of compounds on the cardiovascular, respiratory, and central nervous system functions before the first administration to humans. Chapter 9 describes a manual patch-clamp technique used to study the effect of compounds on the HERG cardiac K⁺ channel in order to evaluate the potential to induce “torsades de pointe”, an arrhythmic disorder that can be fatal in humans.

When unexpected toxicity arises during these studies, it is important to investigate the mechanisms of toxicity and assess the potential translation to humans. Traditional histopathological examination of target organs and clinical pathology parameters are sometimes in default, and novel ‘omics technologies, such as transcriptomics, proteomics, and metabonomics could allow to generate new hypotheses on the mechanisms of toxicity. Detailed protocols related to these ‘omics technologies are presented in Chaps. 10–12.

Of note, the gene expression results obtained via transcriptomics experiments need to be confirmed by quantitative RT-PCR. However, accurate interpretation cannot be performed without proper statistical analysis of RT-PCR data. Chapter 13 examines some of the issues concerning RT-PCR experiments that would benefit from rigorous statistical treatment.

In vitro functional assays can be used to elucidate mechanisms of toxicity in the context of drug safety evaluation. Chapter 14 describes an *in vitro* assay used to evaluate the effect of compounds on the mitochondrial respiration chain in cultured rat hepatocytes. Mitochondrial dysfunction is indeed a major mechanism, whereby drugs can induce liver injury and other serious side effects, such as lactic acidosis and rhabdomyolysis, in some patients. *In vitro* assays can also be used during the early phase of drug development to screen compounds for their potential to induce developmental toxicity. This is illustrated with the Fetax and the zebrafish models in Chaps. 15 and 16, respectively. Drug-induced toxicity is often associated with the formation of reactive metabolites that bind covalently to proteins. Chapter 17 describes *in vitro* assays used at the lead optimization stage of drug discovery to evaluate the potential of drug candidates to bind covalently to proteins by incubating a radiolabeled analog of the compound with liver microsomal preparations or whole cells. Sophisticated mass spectrometry-based methods can also be used to identify chemical-adducted proteins both *in vitro* and *in vivo*. This is illustrated with specific examples in Chaps. 18–21.

Another developing field in drug safety evaluation is the identification and qualification of novel safety biomarkers that can be used to better monitor potential toxicity in both preclinical and clinical studies. Ideally, these new safety biomarkers should be more sensitive and/or specific than the traditional clinical pathology parameters and should be measurable in accessible fluids, such as plasma and urine. Chapters 22–24 provide sophisticated methods to discover new safety biomarkers using proteomics and metabonomics approaches. A protocol to quantify potential protein safety biomarkers by mass spectrometry is also described in Chap. 25.

I would like to thank all the contributing authors for providing state-of-the-art procedures, detailed protocols, and tips and tricks to avoid pitfalls. I am grateful to the series editor, John Walker, for inviting me to edit this volume. The result is a compendium of analytical technologies, including some review chapters, with a focus on clarity and applicability in real life laboratory practice. The intended audience mainly consists of pharmaceutical scientists, toxicologists, biochemists, and molecular biologists, and anyone else with a specific interest in methods used in drug safety evaluation that could be translated to other disciplines.

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