Drug Discovery and Evaluation: Pharmacological Assays

H. Gerhard Vogel (Ed.)

Drug Discovery and Evaluation: Pharmacological Assays

Third Completely Revised, Updated and Enlarged Edition

With 5 Figures and 66 Tables

Springer

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Preface to the Third Edition

The third edition of the book *Drug Discovery and Evaluation. Pharmacological Assays* is presented here.

The volume of data has risen considerably compared to the second edition. In particular, a large number of assays have been added. Meanwhile, data on Safety Pharmacology have been removed from this edition. An extended discussion of that important aspect of pharmacology was published in the book *Drug Discovery and Evaluation. Safety and Pharmacokinetic Assays*, edited by H. G. Vogel, F. J. Hock, J. Maas, and D. Mayer, by Springer in 2006.

Several of my colleagues provided essential contributions to this edition. In particular I am indebted to S. A. Mousa for rewriting the chapter Pharmacological Assays in Thrombosis and Hemostasis, S. G. E. Hart, for rewriting the chapter Activity on Urinary Tract, G. Müller for rewriting and amending the chapter Antidiabetic Activity, B. Schultz for amending the chapter Ophthalmologic Activity, and J. Sandow for rewriting the chapter Endocrinology. All chapters have been revised and thoroughly updated as well.

The approach to drug discovery is changing continuously. Decades ago, many drugs were found by serendipity in clinical trials. Most new drugs, however, were found by the classical approach in animal experiments. This approach has the advantage of relatively high predictability, but it also has the disadvantage that little information is provided about the molecular mechanisms involved in the observed effects, and the detection of drugs with new mechanisms always required new models.

It is generally believed that the costs of developing new pharmaceutical drugs are exploding, while the output of new drugs is actually decreasing. A change in paradigm, the *target-based or mechanism-based drug discovery approach*, was therefore welcomed with great enthusiasm. The techniques of combinatorial chemistry could generate thousands of compounds to be tested against thousands of targets using high-throughput and ultra-high-throughput technology with tremendous capacity. This made it highly effective for the identification of target-selective compounds. However, despite the fact that this approach is very advantageous from a scientific and practical viewpoint, it did not translate into a high success rate in the discovery of new drugs. This has naturally led to questions regarding the success of target-based drug discovery and, more importantly, a search for alternatives.

The target-based approach has therefore been replaced again by the *physiology-based approach*, the classical drug discovery paradigm, or the *function-based approach*, which seeks to induce a therapeutic effect by normalizing a disease-specific abnormality.

On top of this, important changes in the management of some of the larger drug companies have taken place in recent years. Modern managers were installed as chief executive officers and other high-level executives, quite often with little or no biological and technical experience. They were more interested in blockbusters

and in shareholders' value than finding new drugs, especially for rare diseases. Research workers were confronted with cumbersome and inflexible organizational structures characterized by regimentation, control, conformity, and excessive bureaucracy. Consequently, creativity and productivity decreased in this environment. Recently, however, there are signs that the situation is changing.

In this book we ask the question: "Quo vadis, pharmacology?" We suggest giving pharmacologists the freedom, time, and money to carry out their own ideas. We also describe animal models of rare diseases that enable the medically trained pharmacologist to find appropriate drugs.

We are well aware that the rapid progress in biology will once again change the methodological approach in the coming years, and that electronic media will continuously help the researcher to access and share information. However, it is becoming more and more evident that many young pharmacologists have only limited training in classical pharmacological methodologies. When searching for these methods, researchers will only find insufficient information on the methodological details in the electronic databases currently available. To this end, we hope the current book may bridge this gap by comprehensively covering those pharmacological methods utilized for over more than a hundred years.

At this point I would like to express my sincere thanks to all colleagues who contributed to the new and to the earlier editions of this book. Their names and affiliations are given in alphabetical order.

Spring 2007 *H. Gerhard Vogel*

Preface to the Second Edition

The first edition of "Drug Discovery and Evaluation – Pharmacological Assays" has been well accepted by a broad readership ranging from experienced pharmacologists to students of pharmacology. Therefore, already after a short period of time the question for a second edition arose.

The first edition was mainly centered around the personal laboratory experience of the editors and the contributors. The second edition tries to close evident gaps. The input of biochemistry to pharmacology has grown. Molecular pharmacology puts more emphasis on the mode of action of drugs, albeit it becomes clear that the activities of most drugs are not confined to one single mode of action. Studies in single cells become more and more popular, however, they do not cover the complexity of a whole organism. Possible side effects of drugs can be better detected in whole animals than in single cells. Therefore, the new requirements of the health authorities on safety pharmacology put emphasis on experiments not only in whole anesthetized animals but in conscious ones. The second edition of this book takes note of these requirements and devotes special chapters for each indication to safety pharmacology.

Molecular biology also introduced new methods to pharmacology, such as the polymerase chain reaction (PCR), reverse PCR, Northern, Western and Southern blotting. Very recently, microarray technology, proteomics, and mass spectroscopy were added as novel in vitro methods. Furthermore, genetically modified animals have been created which resemble human diseases. Pharmacogenomics has already begun to influence pharmacology and even will have greater input in the future. Special attention is given to these new achievements in various chapters of the book.

The editor and the co-editors are well aware that the rapid progress in biology during the next decades will change the methodological approach. Electronic media will help the researcher for continuous information. However, it becomes more and more evident, that young pharmacologists have only insufficient training in the classical pharmacological methodology. Searching for these methods, e.g., for safety pharmacology, the researchers cannot find sufficient information on the methodological details in the electronic data bases currently available. This hook covering the pharmacological methods of more than hundred years may be of help.

The guidelines concerning the care and use of laboratory animals have been updated.

A change in paradigm of pharmacological research has been claimed but superiorities of these new approaches compared to the old ones have still to be proven. To address this, an introductory chapter on new strategies in drug discovery and evaluation has been added, including combinatorial chemistry; high throughput screening, ultrahigh throughput screening and high content screening; pharmacogenomics, proteomics and array technology. Some critical thoughts on errors in screening procedures have been added.

At this place, we would like to express our sincere thanks to all colleagues who contributed to the new and to the first edition of this book. Their names and their positions are given alphabetically.

March 2002

H. Gerhard Vogel
Also in the name of the co-editors

Preface to the First Edition

This hook is intended to be an aid for experienced pharmacologists as well as for newcomers in the field of experimental pharmacology. The student in pharmacology, the pharmacist and the medicinal chemist will find a survey of pharmacological assays that can be used for a given indication and for which methods have demonstrated their relevance. The researchers working in special fields of pharmacology will find assays in other, unfamiliar areas which might help to expand their own research.

Certain therapeutic domains, such as cardiovascular, respiratory and renal disorders, psychiatry and neurology, peripheral nerve function, pain and rheumatic diseases, metabolic and endocrine diseases including diseases of the gastrointestinal tract, are discussed in this hook.

Each chapter is divided into pharmacological classes, e.g., anxiolytics, antiepileptics, neuroleptics, antidepressants, or anti-Parkinson drugs. For each class, in vitro methods, tests on isolated organs and in vivo methods are described.

For each method the purpose and rationale are given first, followed by a description of the procedure, evaluation of the data, modifications of the method described in the literature, and the relevant references. If possible, a critical assessment of the method based on personal experience is added. The hints for modifications of the method and the extended reference list will be of value for the experienced pharmacologist.

A few words for the justification for a book of this kind: In 1959, A.J. Lehman, Director of the Division of Pharmacology at the Food and Drug Administration, USA, wrote:

> ... Pharmacologists are individualists. Like most scientists they are seldom willing to copy each other's techniques in detail, and so their methods vary from one to the other. Nevertheless, there are basic principles and techniques which must be applied to establish the safety of a new drug.

Visitors could also read a sticker in his office:

> You too can learn pharmacology, in only three lessons: each of them lasting ten years.

Pharmacologists have always used methods from neighboring disciplines; in the past, e.g. from anatomy, pathology, surgery, zoology and predominantly physiology. Useful methods also came from electrophysiology and the behavioral sciences. Earlier drug discovery was almost exclusively based on animal experiments, clinical observations and serendipity.

In recent years, a major input has come from biochemistry. The effect of many drugs in human therapy could be explained biochemicaly as effects on specific enzymes or receptors. With the detection of more and more receptor subtypes, the

activity spectrum of a single compound became more and more complicated. At present, molecular biology provides pharmacologists with human receptors and ion channels expressed in mammalian cells in culture. This avoids the apparently existing species differences, hut the multitude of natural and perhaps artificial subtypes raises the question of physiological and pathological relevance.

The challenge for the pharmacologist always will be to correlate in vitro data with in vivo findings, bearing in mind the old saying: "*In vitro simplicitas, in vivo veritas*". The effects found in tissue cultures are quite often not typical for an intact organism.

Pharmacologists, especially in industry, have the task to find new drugs for human therapy by using appropriate models. Pharmacological models have to be relevant, that means they should predict the intended therapeutic indications. A pharmacological model can be considered relevant or correlational, if the effects obtained correlate with results observed in human therapy.

To be relevant or "correlational", a model has to fulfill some basic criteria:

- First, the model must be sensitive in a dose-dependent fashion to standard compounds that are known to possess the desired therapeutic property.
- Second, the relative potency of known active agents in the model should be comparable to their relative potency in clinical use.
- Third, the model should be selective, i.e. the effects of known agents in this therapeutic indication should be distinguishable from effects of drugs for other indications. Positive data with a new compound allow the prediction of a therapeutic effect in patients.

If new assays are applied to indications for which no effective drug is known, there must be sufficient evidence that this model is relevant for the pathological status in this indication.

The methods presented in this book have been selected according to these criteria.

Considerable discussion is going on about the necessity of animal experiments. One has to accept that only the whole animal can reflect the complexity of a human being. Even an experiment with human volunteers is only a model, albeit a highly relevant one, to investigate therapeutic effects in patients. The degree of relevance increases from isolated molecules (e.g. receptors or enzymes) to organelles, to organs up to conscious animals and human volunteers.

Without any doubt, animal experiments are necessary for the discovery and evaluation of drugs. However, they should be performed only if they are necessary and well conceived.

In Chapter N, regulations existing in various countries concerning the care and use of laboratory animals are listed. Furthermore, guidelines for anesthesia, blood collection and euthanasia in laboratory animals are given. In carrying out animal experiments, one must adhere strictly to these guidelines. Following these rules and planing the experiments well, will eliminate or minimize pain and discomfort to the animal. The methods described in this hook had the welfare of the animals as well as the benefit of the procedure for the well-being of mankind in mind.

Here, we would like to express our sincere thanks to all colleagues who contributed to this book. Their names and positions are given alphabetically below.

Autumn 1996

H. Gerhard Vogel,
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Volume 2

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