DRUG METABOLISM IN DRUG DESIGN AND DEVELOPMENT

Basic Concepts and Practice

EDITED BY

DONGLU ZHANG

MINGSHE ZHU

W. GRIFFITH HUMPHREYS

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PREFACE

Information on the metabolism and disposition of candidate drugs has become a critical part of all aspects of the drug discovery and development process. This comprehensive involvement of drug metabolism information has been brought about by a desire for quality design at an early stage, sometimes referred to as designing good ''developability'' characteristics, and then to work proactively with clinical and safety organizations to impact the design of the various development programs. This desire is driven by the need to reduce attrition rates as a means to effectively lower the cost of drug development.

Drug metabolism information in the early stages of discovery can help guide medicinal chemistry efforts toward optimization of preclinical safety and efficacy properties. This approach can be made even more effective with the active involvement of other disciplines such as pharmaceutics and toxicology. Candidates can be optimized by examining a variety of parameters beyond potency and efficacy. During the development stages drug metabolism information can help guide drug–drug interaction and special population clinical studies. Metabolism information is also critical for designing toxicology studies to that ensure the safety of metabolites is adequately tested and can also be a key part of addressing whether toxicology found in animals is likely to translate to humans.

Drug metabolism, as practiced in the pharmaceutical industry, is a multidisciplinary field that requires knowledge of analytical technologies, expertise in mechanistic and kinetic enzymology, organic reaction mechanism, pharmacokinetic analysis, animal physiology, basic chemical toxicology, preclinical pharmacology, and molecular biology. Scientists entering the field from academia often receive coursework in many of the above areas, but have usually focused the bulk of their research efforts on only one of above mentioned fields. It often requires a number of years of practice for a new scientist to gain a comprehensive understanding of all the disciplines necessary to apply drug metabolism knowledge effectively to the drug discovery and development processes.

This book offers background information as well as practical descriptions of what happens during the drug design and development process. Emphasis will be

placed on issues such as what data are needed, what experiments and analytical methods are typically employed, and how to interpret and apply data. The chapters of this book will highlight facts, detailed experimental designs, applications, and limitations of techniques.

The book was not intended to be a collection of individual reviews, rather a coherent integration of all relevant background information as well as detail of the experimental strategies and processes necessary for drug metabolism research during drug design and development. Authors aimed at providing a balanced, comprehensive perspective on their subject matter and were encouraged to include a full range of experimental approaches. The book contains four parts that should serve to integrate the entire process: Part I, Basic Concepts of Drug Metabolism; Part II, Role of Drug Metabolism in Pharmaceutical Industry; Part III, Analytical Techniques in Drug Metabolism; Part IV, Common Experimental Approaches and Protocols. This structure should provide a valuable resource to researchers seeking to broaden their knowledge of drug metabolism science as practiced in the modern pharmaceutical industry.

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PART I

BASIC CONCEPTS OF DRUG METABOLISM

1

OVERVIEW: DRUG METABOLISM IN THE MODERN PHARMACEUTICAL INDUSTRY

Scott J. Grossman

1.1 INTRODUCTION

It is interesting to contrast contemporary pharmaceutical biotransformation with that practiced by R.T. Williams. The fundamental objectives are virtually unchanged, to characterize the disposition of a drug in animals. In addition, then and now the routes of excretion and overall molecular transformation are still, arguably, the most important aspects of the discipline. However, in the intervening years the scope of technological advancement, scientific breadth of knowledge, and range of impact has expanded in a manner that could not have been foreseen. This chapter will give an overview of biotransformation as it is practiced in the pharmaceutical industry today.

The role of any pharmaceutical biotransformation scientist is to characterize the disposition of a drug to relate this to overall safety and efficacy. The range of information needed to characterize overall disposition is so broad that it is unlikely any single scientist will accomplish the entire *characterization* alone. However, it is critically important that the entire disposition process is thoroughly understood, and then intelligently integrated with other pertinent aspects of the drug's behavior. The history of contemporary pharmaceutical industry is replete with examples of how the lack of fundamental scientific knowledge (e.g., mechanism and effects of enzyme induction), appreciation

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of known metabolic effects (e.g., metabolic activation to toxic reactive metabolites), or incomplete integration of existing information (e.g., drug–drug interactions) led to drastically adverse outcomes. It could be argued that proper integration of information is both more difficult and important than the process of collecting the data itself. Thus, the challenge to the scientist today is to be able to comprehend decades of scientific knowledge, master an array of sophisticated technology, and integrate a diverse range of information to form a sound understanding of a drug's ultimate clinical behavior.

1.2 TECHNOLOGY

There is now an awe-inspiring array of technology available to aid the study of drug disposition. Consider that what once may have taken Williams nearly 6 months to accomplish, might only take about 20 min for a contemporary biotransformation scientist. This modern armamentarium has done much to integrate the power of biotransformation into pharmaceutical discovery and development. However, this tremendous evolution in technology presents its own set of dilemmas.

Taking full advantage of any technology requires an understanding of the technology itself. Fortunately, software and hardware engineering have greatly simplified common use of very sophisticated technologies. The LC/MS/MS instrument today is as common as the HPLC diode array UV instrument 15 years ago. This easy accessibility was greatly facilitated through robust instrument design and great software engineering.

Increasingly, the dilemma is not so much instrument access, as it is a thoughtful choice of exactly what experimental approaches and technology should be chosen to answer the question at hand. The biotransformation scientist is obliged to stay aware of technological innovations of all sorts, including instrumentation. However, the ultimate challenge should always be how to answer the most critical questions in the soundest way. True mastery of technology allows the scientific approach to follow naturally. The temptation to throw technological "sleights of hand" at a problem is often hard to resist.

Every technology has its inherent limits. Often, the specificity that enables prodigious sensitivity can also be a powerful filter of other important information. A rigorous biotransformation scientist is able to stand back and thoughtfully interrogate the strength of her own conclusions, including the technological blind spots of the approach. With thoughtful consideration, complementary technology may be applied judiciously to either flesh out a previous area of ambiguity or address the question from an entirely different perspective. In either case, scientific credibility is served well.

1.3 BREADTH OF SCIENCE

1.3.1 Chemistry

Biotransformation is fundamentally a chemical process. Likewise, the most frequently employed and valuable studies make heavy use of analytical and bioorganic chemistry. Over time, the underlying technology has become sufficiently complex that subspecialization in individual analytical techniques is common. For example, nuclear magnetic resonance spectroscopy (NMR) is invaluable for many unambiguous metabolite structural assignments. In most pharmaceutical companies, NMR specialists are employed to completely master the various facets of the technology. In many cases, these scientists will create sophisticated coupling and decoupling sequences to provide highly specific structural information. Often, their training also makes them most qualified to interpret all forms of NMR spectroscopic data. However, the "complete" biotransformation scientist will, at a minimum, know how to employ NMR spectroscopy to advance their structural understanding of a metabolite. Increasingly, the use of heteronuclear decoupling experiments is considered almost routine in the art.

Furthermore, biotransformation scientists are often fully capable of interpreting the spectra to deduce structure and are also able to recognize when such spectra still leave absolute structural assignments tentative. When one then considers the broader range of additional spectroscopic and chromatographic techniques employed in biotransformation studies, one soon recognizes the degree of technical sophistication required to be an effective biotransformation scientist.

Often, the definitive elucidation of a molecule's metabolic pathway is considered the ultimate goal of biotransformation studies. Proper application of analytical techniques, for the most part, will often be sufficient to achieve this goal. However, as often as it is "good enough" to simply define *what* has happened to a molecule, there are probably twice as many instances where it is also important to understand *how* these changes happened. The best biotransformation scientists are usually good "electron pushers." That is, their knowledge of bioorganic chemistry allows them to understand the mechanism of the molecular rearrangements taking place in each biotransformation process. They are able to both rationalize most biotransformations in a mechanistic sense and recognize when a proposed metabolite structure seems untenable. It is not uncommon to encounter a set of spectroscopic data that seems quite inconsistent with the parent molecule. In these cases, the fundamental principles of bioorganic chemistry are employed to rationalize putative structures that would be consistent with the data.

Increasingly, the roles of medicinal chemists and biotransformation scientists intersect in the discipline of bioorganic chemistry. Frequently, they share a mutual interest in decreasing metabolic liability through structural modification as well as avoiding creation of reactive metabolites

through informed molecular design. Fortunately, their common understanding of bioorganic chemistry also greatly facilitates the intelligent redesign of structures to mitigate these liabilities. At its best, this requires the best of both disciplines and each scientist can develop a deeper fundamental understanding of the other's craft.

1.3.2 Enzymology and Molecular Biology

Although each of these disciplines could be discussed separately, for the contemporary biotransformation scientist these areas are intimately intertwined. Since biotransformations are enzyme mediated, complete understanding of xenobiotic disposition is only achieved when one also considers the role and impact of the individual enzymes involved.

Enzymological techniques allow the study of individual enzymatic reactions as well as the role of individual enzymes in complex systems. Each of the questions "What happens?" "What enzymes contribute?" "How does it happen?" will require separate techniques. It is not unusual to ask and answer these questions in a very short period of time. This obviously requires a certain degree of breadth, versatility, and flexibility along with a fundamentally strong understanding of the literature.

Cells and subcellular fractions from humans and many preclinical species are readily available. These reagents make it possible to make interspecies extrapolations easily. At one time, a major reason cited for early drug attrition was pharmacokinetic failure, attributable to the difficulty in extrapolating pharmacokinetic behavior from animals to humans. In this author's experience, unexpected pharmacokinetic performance in humans is now a rare event. In addition, it is now commonplace to obtain very mechanistic information revealing the probability of observing quite specific molecular events (e.g., toxicity) in humans (Mutlib et al., 2000).

While the availability of trans-species enzyme systems has had a major impact, advances in molecular biology have also enabled the query of increasingly sophisticated questions. Molecular biological methods have made it possible to clone and express enzymes to study reactions at a molecular level. This has improved our ability to study enzyme reactions at a fine molecular level, to discern the contributions of individual enzymes in complex systems, and even to employ them as "bioreactors" to generate small quantities of metabolite standards.

The basis for many metabolizing enzyme polymorphisms is becoming better understood, allowing one to anticipate potential interindividual disposition differences. Molecular biological techniques have defined the basis for polymorphisms and have described the distribution of the variants in a population. It is now quite easy to discern whether a drug may behave differently in one individual compared to another and to even exclude anticipated poor responders from trials in a controlled fashion (Murphy et al., 2000).