PART I

BASIC CONCEPTS OF DRUG METABOLISM
OVERVIEW: DRUG METABOLISM IN THE MODERN PHARMACEUTICAL INDUSTRY

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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
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).
The means by which enzyme systems are regulated are now being appreciated and studied in a mechanistic fashion. Tools available today make it possible to screen against enzyme inducers as well as inhibitors in a relatively inexpensive, well-defined fashion.

1.4 IMPACT OF DRUG METABOLISM ON EFFICACY AND SAFETY

Even in the simplest case, a drug that is injected intravenously and excreted completely unchanged in the urine, there are likely important implications to the human risk/benefit evaluation. Is the excretion so fast that efficacy is compromised? Will the dose need to be adjusted in patients with compromised renal function? Will high drug concentrations in the urinary tract lead to important safety concerns? The biotransformation scientist who only asks “What is happening?” without “What could it mean?” is missing an opportunity to play a larger role in making important decisions. In fact, it can be argued that the biotransformation scientist is perhaps best suited to raise these concerns and is neglecting a critical aspect of their profession by not leading these discussions.

1.4.1 Efficacy

At the earliest stages of drug discovery, an important transition must be made from the screening well to the functioning cell. Even at this stage, there are often significant hurdles related to biotransformation. At every step along the way to higher levels of biological organization, biotransformation inevitably imposes further challenges to the goal of therapeutic efficacy. Understandably then, significant time and resource in drug discovery is spent optimizing a molecule’s disposition properties. Perhaps it is more precise to say that much effort is put into the overall process of molecular optimization to yield a molecule with acceptable disposition properties. This distinction, though subtle, is critically important. For once a molecule is made, its properties are cast and its biological fate cannot be changed. Thus, it is critically important in drug discovery to get the optimization done right.

Few would argue that molecular optimization to achieve adequate pharmacokinetic properties is a high priority in early discovery. Practically speaking, much of this work could be accomplished with little biotransformation insight. By using in vitro and/or in vivo models, a chemistry team may certainly achieve the necessary degree of optimization. However, even when the optimization comes as a result of a well-developed sense of SAR, one recognizes that substantial amounts of intuition and good fortune were also necessary. Luck is fleeting and intuition has its limits. This is particularly true when there is little baseline data and the problem is complex. Thus, a purely empirical pharmacokinetic approach is not likely to be the most efficient path for success.
Pharmacokinetic optimization can be greatly aided with further biotransformation information. A limited disposition study can be extremely useful. Simply looking for intact drug in urine and bile, one may be able to discern significant clearance by renal or biliary excretion. Neither of these disposition routes are normally modeled by high throughput in vitro clearance assays. One may quickly learn that information from in vitro screening is not likely to have the desired benefits. Unfortunately, given current state of knowledge of transporter ligand affinity, screening in these instances is likely to remain a largely “black box” screening effort with in vivo models.

A simple study designed to identify biotransformation “hot spots” is frequently invaluable during pharmacokinetic optimization. Samples from either in vitro or in vivo studies analyzed by HPLC with parallel UV/MS detectors can often quickly identify those aspects of a chemotype most susceptible to metabolism. Now the challenge becomes an exercise of molecular modifications, informed by knowledge of the area of the molecule needing attention.

1.4.2 Safety

By definition, xenobiotic metabolism considers how an organism disposes of a foreign chemical. It is the study of what the body does to the drug. Whether intentional or unintentional, these xenobiotics often have physiological effects. Thus, a major role for biotransformation is to understand how metabolic processes terminate or limit desired physiological effects (efficacy) as well as how other processes may lead to unintended consequences (toxicity).

A drug’s duration of action, its intensity of action, and interindividual variability in responsiveness are frequently related to its disposition properties. For drugs with a narrow therapeutic index, these sources of variability can and do lead to adverse effects and may significantly limit the full therapeutic usefulness of the product. Likewise, drug–drug interactions also lead to unintended effects. As an inhibitor or inducer of enzymes involved in the disposition of other co-medications the drug may cause exacerbated pharmacological effects (inhibitors) or therapeutic lapses (inducers). Again, drugs of this nature may have severely restricted use, depending on the therapeutic utility and the co-medication environment in which they would be used. Thus, without even considering how a drug is metabolized, safety can be affected.

Dr. James Gillette, the Millers, their coworkers and colleagues, and generations after them have documented how molecular biotransformation leads to toxicity (Brodie et al., 1971; Miller and Miller, 1955). Molecular activation (or biological reactive intermediates) is one of the most intensively studied aspects of both drug metabolism and toxicology. Thousands of publications have documented the breadth of reactions leading to reactive metabolites, and thousands of others have shown the breadth of impact throughout the body and among all species. Consequently, there is a
well-developed basis for anticipating structural features that may predispose a molecule to form reactive metabolites. Once discovered, reactive metabolites can often be avoided or minimized by judicious molecular redesign. In fact, both biotransformation scientists and medicinal chemists are obligated to know this area. This knowledge facilitates design of molecules without known liabilities, or at least guides the incorporation of certain worrisome features in a way that can be carefully evaluated.

Perhaps because of the well-developed literature linking biotransformation and toxicity, there seems to be a widely held perception that “most toxicity is due to metabolism.” This author does not subscribe to that thesis and will not discuss it further here (Grossman, 2006). However, xenobiotic-induced toxicity is a substantial issue to be dealt with. By most accounts, toxicity is the single most common cause for drug attrition. It is inconceivable that a contemporary pharmaceutical biotransformation scientist will not be involved in toxicity-related investigations in their career, and probably will be involved many times. However, it is human nature to view everything as a nail if you are a hammer. The most tempting course of action for a biotransformation scientist is to “start with the molecule” and posit putative reactive metabolites that could give rise to the observed effects. An alternate approach is to “start with the lesion” and query the pathophysiological drivers that give rise to observed effects. This would include the consideration of unanticipated interactions of the parent molecule or its stable metabolites with any of the 40,000 gene products expressed in the affected organism. Either approach, applied with prudence and substantial good fortune, can yield the answer. With maturity and discipline, the biotransformation scientist learns to dissect toxicology issues through the scientific method, proposing hypotheses and carefully designing experiments to eliminate false hypotheses in a definitive fashion.

1.5 REGULATORY IMPACT AND IP POSITION

Understandably, the majority of biotransformation scientists would not readily consider intellectual property a fundamental aspect of their work. Likewise, the regulatory impact of findings (or their lack of study) is infrequently thought of in the normal course of work. However, it is inarguable that if a drug is not registered there will be no derived therapeutic benefit. Without therapeutic benefit, the entire discovery and development endeavor is simply a very, very expensive set of experiments.

Registration and marketing approval of a new chemical entity (NCE) is increasingly difficult. In short, the era of the contemporary pharmaceutical industry has provided a long list of “lessons learned” from the accumulation of good and bad experiences. The inevitable evolution and improvements derived from this have benefited society with more efficacious and better tolerated drugs. As a consequence, the formalization of these “lessons learned” has created a seemingly incomprehensible list of points to consider,
document, and evaluate in the risk/benefit decision-making process. Global regulatory bodies are charged with promulgating these various considerations through guidances and regulations. Further, it is their responsibility to act as an advocate for the consumer, wherein a consistent set of practices will be applied to form a judgment regarding benefit versus risk, devoid of bias from financial considerations. Within this arena, the complete body of a drug’s study must be summarized intelligently such that all pertinent facts are properly accounted for and put into perspective.

If one now considers this final full evaluation as the ultimate basis for a drug’s approval, it becomes clear that one must take additional steps to evaluate the data one produces. In short, one really needs to ask “I know I like what the data say, but will someone else accept the data as readily as I did and see it in the same light?” One must ensure that the proper scope of data has been collected (to meet regulatory standards). The data must be collected and documented in a proper fashion (to meet GLP requirements) and attain a proper degree of precision and accuracy in the samples studied (to weigh the value of derived conclusions). Information must be integrated and described in a fashion that tells a compelling story, and importantly, it must be looked at from many different perspectives to ensure that there are no holes or flaws that undermine important decisions. In the end, none of this is done without consistently good science. But one must also recognize that substantial good judgment and perspective is necessary that comes only through experience.

If the drug application is successful, the pharmaceutical sponsor must create a “label” that guides the physician’s prescribing practice. The statements and claims in a label are negotiated between the pharmaceutical sponsor and regulatory agencies. Ultimately, the label is the physician’s most accessible source of information describing the drug’s various properties and how these properties may affect the drug’s impact on patients. As mentioned previously, knowing what the body does to the drug can often be as valuable as knowing what the drug does to the body. A well-written label should give a concise understanding of the drug’s disposition properties, particularly when dispositional characteristics may result in widely varying responses among patients. Given the frequency of drug registrations in any individual’s career, the creation of the product label may be the ultimate pièce de résistance for the biotransformation scientist. It is likely their last chance to leave an indelible mark of their work, in this austere summary of many years of investigation.

It should not be overlooked either that label claims are often the basis of marketing advantages. It is quite common that a drug’s dispositional characteristics provide benefit to the patient relative to other agents in the class. Without doubt, this differentiation may play a prominent role in subsequent marketing efforts. Thus, there is a financial imperative that differentiating features be perceived properly, as well as the ethical obligation that they are described accurately.
The cumulative cost of bringing a drug to market is staggering. Without some assurance that these costs will be recovered through eventual sales, it is economically untenable to take on the high risks of failure attendant with drug development. This fiscal assurance is provided, in part, through various patents assigned during discovery and development. Patents covering composition of matter are among the most valuable for a novel drug, as this allows the patent holder exclusive rights for a reasonable period of time. Eventually, much of the

FIGURE 1.1 Examples of biotransformation reactions leading to a preferred marketed drug (Fura et al., 2004).
information learned from the drug’s discovery and development is shared in the public domain. A carefully crafted patent protects the patent owner from having this substantial investment from being misappropriated.

The astute biotransformation scientist will readily recognize that many disposition-related events can affect the propriety of the original intellectual property. Absorption by itself can be a fairly complicated matter. In several cases, a better understanding of the processes affecting absorption has led to better versions of the original discoveries (e.g., prodrugs). Perhaps the most complex aspect of disposition relevant to this topic is biotransformation itself. The range of transformations can range from reasonably subtle chiral inversions to fairly dramatic intramolecular rearrangements. Along this continuum are a range of molecular conformations that may either retain much of the parent molecule’s original attributes, or in many cases be either the actual entity responsible for activity or an improved version of the original. Therefore, it is important to contemplate these events and carefully characterize the range of molecular species derived. The incredible biosynthetic diversity of metabolism enzymes can infer important new structures never envisioned in the original patent claim. If not considered, it is quite possible, or even inevitable, that someone else will recognize this opportunity. Indeed, many prominent drugs are metabolism-related variants marketed by another company (Fig. 1.1).

1.6 SUMMARY

Drug disposition is an incredibly broad discipline. The range of skills and knowledge required to master this discipline is easy to underappreciate. While somewhat daunting in its own right, mastery of the various skills needed to acquire data is the easiest part of the process. The mark of a scientist will always be creating knowledge from data. For most scientists, the most satisfying aspect of their profession is the creation of highly impactful knowledge. This requires the foresight to ask the right questions, collect data in the right way, correctly integrating all of the information, and having the ability to apply this newfound knowledge in a meaningful way. Moreover, the astute scientist recognizes significant unexpected findings and has the perspective to see new and important questions raised in the process.

REFERENCES


REFERENCES


