Drug-Like Properties

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Concepts, Structure, Design, and Methods from ADME to Toxicity Optimization

By

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Dedication

Li Di dedicates this book to:
My parents: I am infinitely in debt to you
My sisters, Ning and Qing: for being my best friends
My children, Kevin and Sophia: I am very proud of you.
Ed Kerns dedicates this book to:
William, Virginia, Nancy, Patrick, Chrissy,
Brian and Lillian: for your love and support.

Preface

PREFACE TO SECOND EDITION

Over the past eight years since the first edition of this book many innovative new drugs have been discovered through the creativity and persistence of drug discovery scientists. These certainly benefit the life quality and length for millions of patients and the joy of their families and friends. It is a privilege to work in this field.

Aiding this effort are the many new developments in pharmaceutical sciences and technology. These give improved understanding of drug delivery, target interaction, efficacy and safety. Many new developments are incorporated in this second edition. They include:

- deeper understanding of *transporters* and their effects on pharmacokinetics and drug-drug interaction
- consensus on *free drug concentration* as the controller of *in vivo* efficacy
- broader understanding of *metabolic enzymes*
- improved *modeling* of human pharmacokinetics for clinical dosing and safety prediction
- new in vitro and in vivo *methods* for improved reliability of measurements of drug physicochemical and ADMET properties
- improved schemes and methods for *blood–brain barrier penetration*
- *toxicity indicators* such as time-dependent inhibition, safety indexes and physicochemical markers of off-target effects

New examples from the medicinal chemistry literature have also been included to illustrate SAR and lead optimization approaches.

Above all, the purpose of this book is to assist drug discovery scientists through a resource that explains the fundamentals, effects and strategies they can apply for selection and optimization of drug discovery leads and clinical planning, to improve success in drug discovery and patient therapy. We have been gratified by the comments of individuals who have indicated that this book assisted them. To all, we wish drug discovery success.

PREFACE TO FIRST EDITION

Drug research is a fulfilling career, because new drugs can improve human health, quality of life and life span. For scientists dedicated to drug research, it can also be a supremely challenging mission, owing to the numerous attributes that must be simultaneously optimized to arrive at an efficacious drug-like compound. ADME/Tox (absorption, distribution, metabolism, elimination, toxicity) is one of these challenges. Of the thousands of novel compounds that a drug discovery project team invents and that bind to the therapeutic target, typically only a fraction of these have sufficient ADME/Tox properties to become a drug product. This book is devoted to providing you, the drug research scientist or student, with an introduction to ADME/Tox property concepts, structure design, and methodology to help you succeed with these challenges.

Chemists will be aided by the case studies, structure–property relationships and structure modification strategies in this book. These assist in diagnosing the substructures of a lead structure that are not drug-like and suggest ideas for ADME/Tox structure design. Overviews of property methods provide the background needed to accurately interpret and apply the data for informed decisions. For ADME/Tox scientists, insights on property assays assist with selecting methods and generating data that impacts projects.

Biologists/pharmacologists will benefit from an increased understanding of ADME/Tox concepts. This is especially important, because in recent years the application of property data has expanded from optimizing *in vivo* pharmacokinetics and safety to biological assays. Low solubility, chemical instability, and low permeability can greatly affect bioassay data. Equipped with this understanding, biologists are better able to optimize bioassays and include property affects in data interpretation.

Accordingly, understanding ADME/Tox is important for all drug researchers, owing to its increasing importance in advancing high quality candidates to clinical studies and the processes of drug discovery. ADME/Tox properties are a crucial aspect of clinical candidate quality. If the properties are weak, the candidate will have a high risk of failure or be less desirable as a drug product. ADME/Tox has become integrated in the drug discovery process and is a tremendous asset in guiding selection and optimization of precious leads. This book is a tool and resource for scientists engaged in, or preparing for, the selection and optimization process. The authors wish you success in creating the pharmaceuticals of the future that will benefit all people.

In preparing this book, the authors had the support and council of many drug research colleagues. The leadership of Magid Abou-Gharbia, Guy T. Carter and Oliver J. McConnell of Wyeth Research, Chemical and Screening Sciences are greatly appreciated. The careful manuscript review and feedback by Christopher P. Miller was highly beneficial. The thoughtful comments of several anonymous reviewers are greatly appreciated. LD thanks Prof. Donald M. Small, Prof. Bruce M. Foxman, Prof. Ruisheng Li for guidance. EK thanks Prof. David M. Forkey, William L. Budde, and Charles M. Combs for mentorship. We thank Prof. Ronald T. Borchardt and Christopher A. Lipinski for their friendship, collaboration, and leadership in the ADME/Tox and medicinal chemistry fields. The enthusiastic feedback of students in the American Chemical Society short course on Drug-like Properties was highly valuable. The collaborative adventure of understanding drug-like properties in drug discovery was shared with numerous Wyeth Research colleagues in Pharmaceutical Profiling and Medicinal Chemistry and their respectful, innovative collaboration is greatly appreciated.

Chapter 1

Introduction

1.1 DRUG-LIKE PROPERTIES IN DRUG DISCOVERY

Drug properties comprise the structural, physicochemical, biochemical, pharmacokinetic (PK), and toxicity characteristics of a compound. Certain values of drug properties are more advantageous for discovering new drugs. This concept advanced over many years. A key article that discussed advantageous property values commented:

> *"Drug-like is defined as those compounds that have sufficiently acceptable ADME properties and sufficiently acceptable toxicity properties to survive through the completion of human Phase I clinical trials." [1]*

ADME is absorption, distribution, metabolism, and excretion, the processes that determine PK. Phase I clinical trials measure human safety and PK. Thus, "drug-like properties" constitute a property profile that is consistent with the drug properties of most commercial drugs.

Drug properties were traditionally a focus of drug development. However, in the 1990s the responsibility of optimizing the drug properties of clinical candidates was given to drug discovery scientists. It has been commented:

> *"…drug-like properties are … intrinsic properties of the molecules and it is the responsibility of the medicinal chemists to optimize not only the pharmacological properties but also the drug-like properties of these molecules" [2]*

Drug properties are an integral part of drug discovery. In the early phase of drug discovery, drug properties are used to select the "hits" that are suitable starting points for research on a new clinical candidate. They serve to focus drug discovery efforts into chemical space that has a higher probability of PK and safety success. Later in drug discovery, they have major influences on understanding structure-property relationships (SPR), guiding structure modifications for property optimization, diagnosing the causes of inadequate PK and toxicity, optimizing and interpreting bioassays, and building prospective models of human PK and its relationship to pharmacodynamics (PD). Medicinal chemists optimize the drug properties of leads in parallel with optimizing efficacy, selectivity, and novelty. This is accomplished by iteratively modifying the structure and measuring the properties of the new compound.

As drug discovery scientists extend the science of PK and toxicity, understanding about drug properties and their complex influence on drug candidates expands. The early focus on lipophilicity, molecular weight, and hydrogen bonding has expanded to complexities of properties, including solubility, permeability, metabolic enzymes, and transporters. The early concept of drug-like property ranges has advanced to multiparameter optimization approaches [3], pharmacokinetic/pharmacodynamic (PK/PD) modeling [4], and physiologically based PK (PBPK) [5]. This mirrors the increasing complexity and sophistication of all aspects of drug discovery, when scientists pursue multiple lines of investigation involving diverse disciplines. The focus is on integration of these disciplines through complex simultaneous studies to optimize and select new clinical candidates with a *balance* of efficacy, selectivity, PK, and safety [6].

One example of the fundamental role of PK and safety in drug discovery is the concept of "three pillars of survival" of drug candidates through Phase II [7]. The pillars are

> *"… the fundamental pharmacokinetic/pharmacodynamic principles of exposure at the site of action, target binding and expression of functional pharmacological activity …"*

Drug properties focus on the first pillar, exposure of the drug at the site of action. Thus, the field has advanced from general characteristics of drugs that succeed to detailed study of the complex physicochemistry and biochemistry that affect human PK and safety and effectively model human clinical outcomes.

Drug-Like Properties. http://dx.doi.org/10.1016/B978-0-12-801076-1.00001-0

1.2 PURPOSE OF THIS BOOK

The various drug properties, terminology, and assays can be overwhelming to drug discovery scientists and students without sufficient introduction. Some texts on drug properties are daunting because they are written from the perspective of experts in pharmaceutics or drug metabolism/PK and contain detail and mathematical equations that are not easy to understand for drug discovery scientists. This book is a practical guide for medicinal chemists, biologists, pharmacologists, and students. It provides background material and real-world, practical examples for practicing drug discovery scientists who need to plan experiments, make sense of complex data, and arrive at informed decisions.

This book also provides tools for working with drug properties. First, the interactions of drug molecules with the in vivo environments they encounter after administration are described, in order to understand why properties limit drug exposure to the therapeutic target. Next, key drug properties are explored (Figure 1.1) in terms of

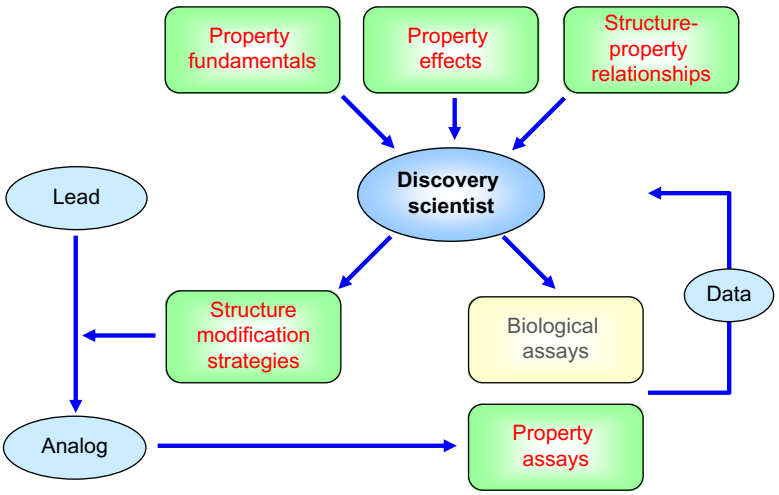

FIGURE 1.1 This book equips discovery scientists and students with a practical understanding of property fundamentals, property effects, and structure-property relationships that can be applied to improving lead series and biological activity. Literature examples of structure modification strategies to improve properties are described for chemists to apply to current projects. Information on property assays provides understanding of the available methods and reliable interpretation of the data.

(1) fundamentals of each property;
(2) effects of each property on PK, safety, and biological experiments;
(3) SPR case studies, to see how structure affects properties;
(4) structure modification strategies, to guide property optimization;
(5) strategies for using the properties to achieve a quality clinical candidate;
(6) effects of properties on in vitro and in vivo biological measurements;
(7) description of property methods, for accurate measurement and application of the data.

These equip drug discovery scientists for increased effectiveness in lead selection, lead optimization, and the enhancement of drug discovery biology and pharmacology assays.

Property-related concepts are described with a minimum of math and emphasis on practical application. Specific property applications in diagnosing poor PK, designing prodrugs, and formulation for in vivo dosing are also discussed.

A scheme for the workflow of this book is shown in Figure 1.1. Drug discovery has diverse elements that must be delicately integrated and balanced. Drug properties are important characteristics that help to achieve a quality clinical candidate.

PROBLEMS

(1) Define the term “drug-like”.
(2) What are two major lead optimization areas in drug discovery?
(3) How can understanding compound properties assist drug discovery biologists?
(4) Compound properties can affect which of the following: (a) pharmacokinetics, (b) bioavailability, (c) IC_{50}, (d) safety?

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Chapter 2

Benefits of Property Assessment and Good Drug-Like Properties

2.1 INTRODUCTION

Drug discovery is continuously advancing as new fundamental knowledge, methods, technologies, and strategies are introduced. These new capabilities result in changes in the drug discovery process. For example:

- Screening for lead structures changed from direct testing in living systems to in vitro high-throughput screening and computational virtual screening.
- Initial leads (hits) for optimization changed from natural products and natural ligands to compounds from large synthetic libraries of diverse structures that cover wide chemical space.
- Information for compound design was enhanced from structure-activity relationships (SAR) to x-ray crystallography, nuclear magnetic resonance binding studies, and computational modeling.
- Lead optimization chemistry changed from one-at-a-time synthesis to parallel synthesis of multiple analogs.
- Traditional sequential experiments changed to parallel experiments, such as automated assays in microtiter plate formats with robotics.

Drug discovery is constantly reevaluating itself in order to advance in speed, efficiency, and quality in order to remain successful.

Pharmacokinetics (PK) and safety assessment and optimization is another area of drug discovery advancement. It offers significant opportunities to enhance drug discovery success. This book focuses on the fundamental knowledge, methods, and strategies for PK and safety, and how structures are optimized to improve these properties. This chapter discusses the benefits of PK and safety optimization.

2.2 DISCOVERY SCIENTISTS OPTIMIZE MANY PROPERTIES

There are many properties that affect PK and safety. Property liabilities often vary between different chemical series. Examples of properties of interest to discovery scientists include the following:

- Structural properties
  - Lipophilicity
  - Topological polar surface area
  - Hydrogen bond acceptors and donors
  - Ionization constant
  - Molecular weight
  - 3-Dimensional shape
  - Reactivity
- Physicochemical properties
  - Solubility
  - Permeability
  - Chemical stability
- Biochemical properties
  - Metabolic stability
  - Transporters

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  - Blood-brain barrier
  - Plasma stability
- Safety
  - Drug-drug interaction (metabolic enzyme, transporter)
  - Reactive metabolites
  - Secondary pharmacology
  - hERG (human ether-à-go-go-related gene) blocking causing ventricular fibrilation
  - Mutagenicity
  - Cytotoxicity
  - Teratogenicity
- PK
  - Clearance
  - Volume of distribution
  - Area under the curve
  - Half-life
  - Bioavailability

The chemical structure determines the structural properties (Figure 2.1). When these interact with the physical environment, they cause the physicochemical properties (e.g., solubility). When these interact with proteins, they produce biochemical properties (e.g., metabolism). Ultimately, interaction within the physicochemical and biochemical environments of living systems determines PK and toxicity.

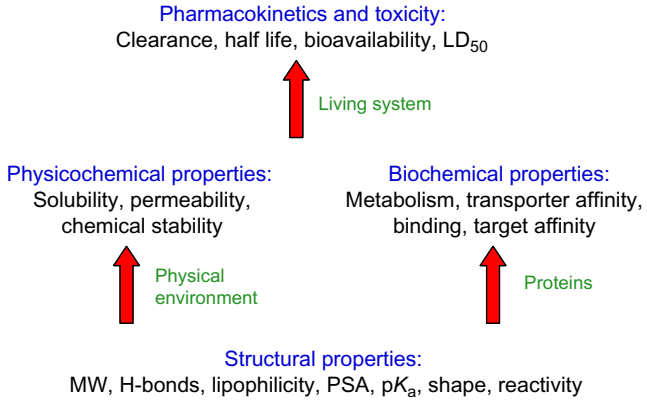

FIGURE 2.1 Chemical structure inherently determines the structural properties, which determine the physicochemical and biochemical properties when the structure interacts with the physical and macromolecular milieu and these determine in vivo PK and safety.

Drug discovery scientists juggle these properties and activity. Medicinal chemists determine the relationship of structure to properties within a chemical series by developing structure-property relationships (SPR), just as for SAR development. They design and enact structure modifications to improve the PK and safety. This is followed by another round of property assessment of the new structural analogs to determine if the desired properties improved while retaining the other properties and activity at a sufficient level.

2.3 INTRODUCTION TO THE DRUG DISCOVERY AND DEVELOPMENT PROCESS

Before exploring how properties affect drug candidates, it is useful to briefly review the process of drug discovery and development. New drug candidates are invented during the drug discovery stage (Figure 2.2). They then enter clinical development and, if approved by the regulatory agencies (e.g., U.S. Food and Drug Administration, European Medicines Agency), become drug products that are used in patient therapy. The major activities in each stage are listed in Figure 2.2. This book focuses on the drug discovery stage. However, the later stages impose stringent drug-like requirements on the properties of compounds in drug discovery. Thus, it is necessary to anticipate these requirements during drug discovery and only advance compounds to development that have the highest chances of success.

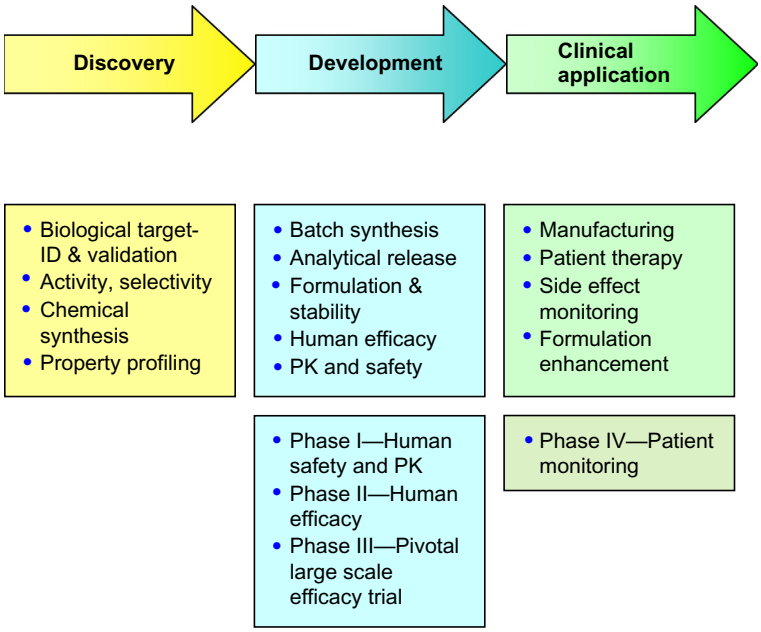

FIGURE 2.2 Overview of drug discovery and development stages with their major activities.

Drug discovery is diagramed in greater detail in Figure 2.3. In general, successive stages involve increasing depth of study and more stringent advancement criteria. The drug discovery screening process initially casts a broad net, to explore diverse pharmacophore structural space. Then it narrows these possibilities to select a few lead scaffolds (templates, chemical series). These are structurally modified to explore SAR, the cornerstone of modern drug discovery, during the lead optimization stage. Finally, candidates for development are subjected to in-depth preclinical studies to qualify them for development.

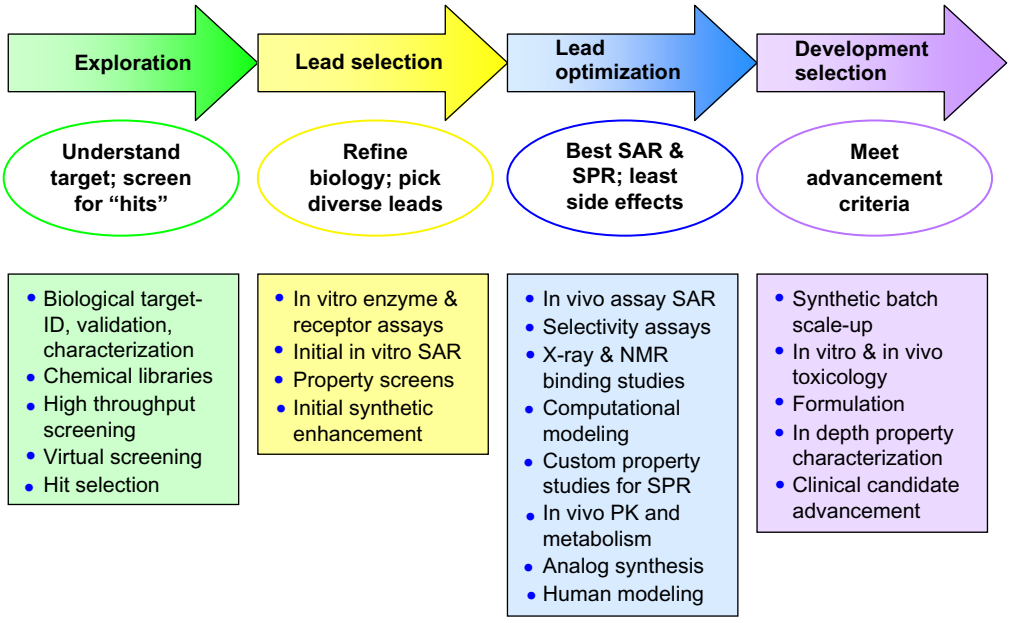

FIGURE 2.3 Stages of drug discovery, primary goals, and major activities.

2.4 BENEFITS OF GOOD DRUG-LIKE PROPERTIES

2.4.1 Reduced Development Attrition

For much of the early history of drug discovery the focus was on finding novel active compounds. Issues such as PK, toxicity, solubility, and stability were addressed during development. In 1988, a pivotal article [1] on the reasons for failure of drugs in development revealed a startling problem. About 39% were failing in development owing to poor biopharmaceutical properties (PK and bioavailability). With the high cost of development, this represented a major economic loss for the companies. Furthermore, years of work on discovery and development were lost and the introduction of a new drug product to patients was delayed.

This great need for enhancement was actively addressed by adding resources to assess properties during late drug discovery. Sorting out the compounds with acceptable properties at this stage did not require the rigorous methods applied during drug development. Thus, for this task, methods used during development were adapted to use fewer resources and operate at higher throughput. Criteria were also relaxed to reflect the reduced accuracy and precision of the revised methods and the lower level of detail needed for decisions at this stage. The assessment of PK was implemented in the late-drug discovery/early-development stage. This testing succeeded in keeping poor candidates from progressing into the drug development pipeline and it reduced development attrition.

2.4.2 More Efficient Drug Discovery

Once this late-discovery property assessment was in place and the attrition burden was reduced for development, it revealed a further drug discovery need. Candidates that were failing in late-drug discovery, owing to poor properties, still caused a great burden on drug discovery. Failure late in drug discovery meant that the project to discover a new drug had lost valuable time and resources on the failed candidate and had to start over. This recognition led to the implementation of property assessment even earlier in drug discovery, so that such losses would be reduced. In pharmaceutical companies, this has been done by a number of different approaches. In one approach, higher throughput animal PK capabilities were added earlier in drug discovery in order to screen more compounds for in vivo PK. This strategy measures the key PK properties that can predict in vivo candidate success (e.g., clearance, oral bioavailability, exposure). In a second strategy, higher throughput in vitro property assays are used. These assays measure the fundamental physicochemical and biochemical properties, such as solubility, permeability, and metabolic stability, which determine PK. in vitro studies require fewer resources and animals per compound than PK studies, thus, more compounds may be assessed using in vitro assays. Also, physicochemical and biochemical properties, measured using in vitro methods, are more specific for a particular property for medicinal chemists to modify and improve [2–4]. Medicinal chemists can more closely correlate these physicochemical and biochemical properties to discrete compound substructures than PK parameters that are influenced by multiple properties. Physicochemical and biochemical methods typically measure a single specific property (e.g., passive diffusion permeability) that can be related to chemical structures and be modified accordingly. Most pharmaceutical companies use a combination of these two strategies during drug discovery.

As a result of these enhancements of drug discovery, the property-related failure of compounds in development declined dramatically from 39% in 1988 to 10% in 2000 [5]. Figure 2.4 indicates that pharmaceutical companies have been

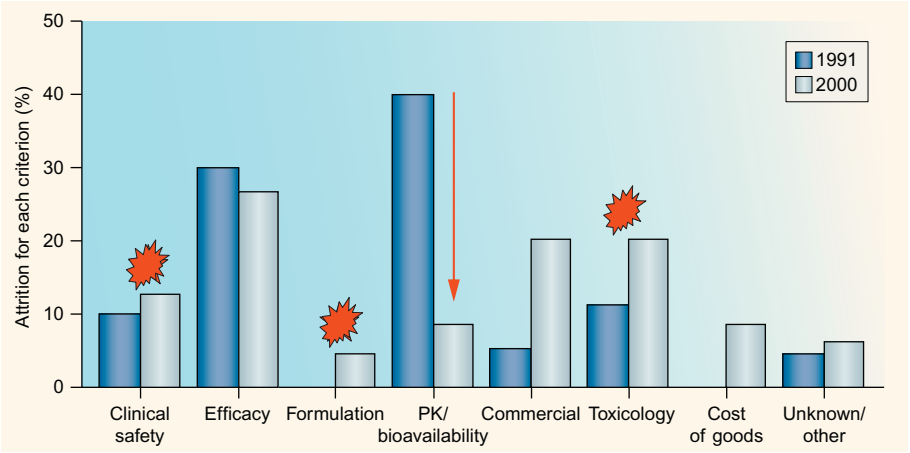

FIGURE 2.4 Between 1991 and 2000 the development attrition owing to PK and bioavailability was greatly reduced. Toxicology, clinical safety, and formulation continue to be significant drug-like property issues [5]. *Reprinted with permission from Kola, I., Landis, J. (2004). Opinion: Can the pharmaceutical industry reduce attrition rates?* Nat. Rev. Drug Discov. 3, *711-716. Copyright 2004, Macmillan Publishers Ltd.*

successful in improving the biopharmaceutical properties of drug development candidates. The 2000 study also suggests that other issues (toxicity, formulation) are continuing challenges.

2.4.3 More Efficient Drug Development

While the rate of candidate failure in drug development has decreased by early termination of candidates with poor properties during drug discovery, some candidates with marginal properties have still progressed into drug development. Even though these might not fail in drug development, they impose significant inefficiencies on development by increasing development costs and prolonging the development timelines.

For example, compounds with poor solubility and stability usually require a longer development timeline and more resources, owing to more difficult formulation development, stability testing, and dissolution studies. Sophisticated formulations can improve solubility, dissolution rate, and compound stability. It is tempting for drug discovery scientists to shift the burden to fix marginal dissolution rate to development pharmaceutics scientists by using sophisticated formulations. While this may be an acceptable choice for first-in-class therapies, for other drug products it can impose a burden on development resources and timeline and delay the introduction of a new drug product.

For a new drug that would produce hundreds of millions of dollars of sales in its first year, $5-10 million of sales are potentially lost for each week of delay in discovery or development. Furthermore, if a patent has been filed, each week of delay could result in one less week of patent exclusivity during the time of highest sales for the inventing company. Thus, there are real economic considerations that drive enhancement of compound quality.

In most cases, it is more advantageous to try to improve drug properties, such as solubility, stability, and permeability, during drug discovery. This is best accomplished by modifying the chemical structure. Modifications are usually performed at sites in the molecule that are shown by SAR to not be critical for therapeutic target activity. In some cases, the structural requirements for ligand binding to the target do not permit structure modifications to improve properties. Under these conditions, drug discovery scientists must decide if the drug candidate still has viability as a drug product. Attention to properties and a workflow that includes property optimization during drug discovery allow for the best chance of discovering a candidate that combines all of the qualities of a successful drug product.

2.4.4 Higher Patient Compliance

Another result of poor properties is that the patient might have to take on a greater burden. For example, if the drug is poorly absorbed, higher doses might be needed to reach the therapeutic levels. The dosing regimen might need to be shifted from oral to intravenous, which is not convenient for dosing among the wide patient population. If the drug has a short in vivo half-life, due to metabolic instability, then it will need to be dosed more frequently. Patients are less likely to consistently self-administer drugs that require higher and more frequent doses per day. Once per day dosing of a solid dosage form by mouth is preferable for a drug product. Also, if a drug is not free of side effects, the patient must endure unpleasant or unhealthy side effects. After a while, the patient might stop taking the drug because the side effects are discouraging. Pharmaceutical companies and academic laboratories have a strong commitment and mission to save and enhance the quality of patient life, thus, patient burdens, needs, and benefits are a primary focus.

It has been commented, retrospectively, that if we had assessed properties in the past, then some of our current drug products that have poor properties (e.g., frequent high doses, side effects) would not have become available for clinical therapy. It is true that some current drugs have poor properties and may not have been approved by the regulatory agencies under current criteria. However, it is widely recognized that early property assessment and optimization provide the opportunity for earlier correction of property limitations. If the current property awareness and assessment had been available at the time of discovery of those drugs with poor properties, then better structural analogs that have comparable potency without the property limitations may have been discovered. In this way, even better drugs might be available sooner and patients would be more likely to take the drug regularly.

2.4.5 Improved Biological Research in Drug Discovery

In addition to development problems, poor properties can also cause problems during drug discovery. Once property data became available during drug discovery, their value to discovery in ways other than PK began to be recognized. We now know that when drug discovery project teams encounter unexplained problems, some of these are due to poor properties [2,6,7]. In the same way that drug-like properties optimize the delivery of drug molecules to the in vivo therapeutic target protein, properties optimize the delivery of drug molecules to the in vitro target protein in a bioassay.

Here are examples of how poor drug properties can reduce the quality of drug discovery biological research:

- Low or inconsistent bioactivity responses for in vitro bioassays can be due to precipitation, owing to low solubility of the compound in the bioassay medium or in dilutions prior to the assay.
- Low activity in bioassays may be due to chemical instability of the compound in the assay matrix.
- When transitioning from enzyme or receptor activity assays to cell-based assays, an unexpectedly large drop in activity can result. This might be due to poor permeability or efflux of the compounds through the cell membrane, which must be penetrated to reach intracellular targets, or lower solubility or stability in the cell-based assay than in the enzyme assay.
- Compounds may be unstable or insoluble in the dimethyl sulfoxide solutions that are stored in microtiter plates and experience freeze-thaw cycles or are exposed to various physicochemical conditions in the laboratory.
- Poor in vivo efficacy for a central nervous system (CNS) drug may be due to poor penetration of the blood-brain barrier.
- Poor in vivo efficacy might be due to low free-drug concentrations in the plasma and target tissue, because of poor PK, low bioavailability, or instability in the blood.

These effects of poor properties may be unrecognized if drug discovery scientists are unaware of their effect and not vigilant to check for the effects or insure that experiments are designed and interpreted to account for the properties. Poor properties can limit exposure of the compound to the target protein in the biology experiment. This property effect might be misinterpreted as actual SAR and a valuable pharmacophore might be overlooked. If the potential effects of poor properties on bioassays are taken into consideration, then the active pharmacophore might be rescued by testing under more appropriate conditions to obtain accurate biological data. Structural modification can then improve the deficient property.

2.4.6 Enabled Partnerships for Drug Development

Drug development is a partnership, in which the development group partners with drug discovery and agrees to apply valuable resources to a clinical candidate invented in drug discovery. This is exemplified by the common strategy whereby drug candidates are discovered by smaller organizations (e.g., biotech companies, academic laboratories, government institutions, nonprofit organizations) and licensed into larger pharmaceutical companies that have development resources. During the "due-diligence" process of candidate review, PK and safety properties are one of the elements that are carefully reviewed. This was not always the case, but when resources were invested because of an interesting activity or efficacy profile and the candidate failed because of inadequate PK or toxicity, the PK and safety due-diligence increased. Therefore, it is valuable to smaller drug discovery organizations to insure that licensing candidates have quality PK and safety properties, in order to get a license that has favorable compensation.

In the same manner, in large pharmaceutical companies, development organizations have the opportunity to choose between clinical candidates discovered by the internal company drug discovery team or by the outside organizations. An internal development partnership is strongest if the internal team has a candidate with a strong PK and safety profile.

2.4.7 Human Modeling and Clinical Planning

The value of computational modeling to provide reliable human PK and safety predictions for decision making during drug discovery and for planning human clinical studies has been widely accepted. The information necessary for such a model is extensive. in vitro drug property values are crucial data for building human models with high confidence.

2.4.8 Balance of Properties and Activity

If the drug discovery project focuses solely on in vitro binding to the active site of the target protein and SAR, the resulting candidate might have an inadequate PK or safety profile. For example, it might be too polar to penetrate the blood-brain barrier to reach the intended CNS target, it might be unstable and be rapidly cleared by first-pass metabolism, or it might be too insoluble to be well absorbed in the intestine. Once nanomolar activity is obtained, it is hard to go back and fix properties by structural modifications, because it may be necessary to modify the substructures that were added in order to enhance binding affinity.

This situation is shown in Figure 2.5. A primary focus on activity can yield compounds that are very effective as ligands for the target protein, but the properties may be inadequate for the compounds to become successful drugs. For example, increased lipophilicity can enhance target protein binding; however, it can also reduce solubility and metabolic stability. A balanced attention to both activity and properties yields candidates that can be good drugs (Figure 2.6). Good activity and drug-like properties are complementary and both are necessary for a good drug product. The most active or selective compound might not make the best drug product, because of property limitations that cause poor PK or safety. A compound with

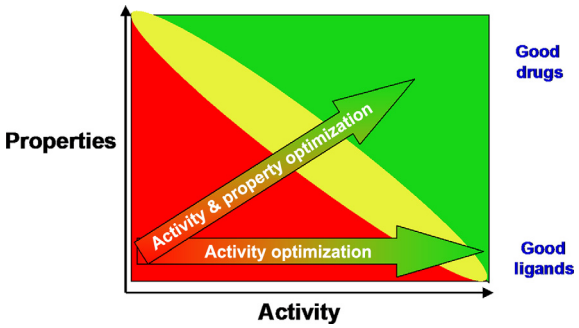

FIGURE 2.5 The strategy for discovering clinical candidates has progressed from a focus on activity to balanced attention to activity and properties [4]. *Reprinted with permission from Kerns, E. H., Di, L. (2003). Pharmaceutical profiling in drug discovery*. Drug Discov. Today 8, *316-323. Copyright 2003 Elsevier.*

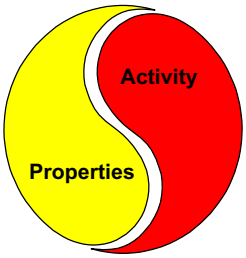

FIGURE 2.6 Pharmaceuticals balance activity and properties.

moderate target binding and good PK and safety properties may produce a better in vivo therapeutic response, safety window, and be a better drug product for patients. As in the sport decathlon, the candidate is tested by many events/challenges and it is the combined performance that determines success, not being the best in an individual event.

The multitude of challenges faced by drug discovery scientists has been variously characterized. One useful image is to characterize them as a series of hurdles that a compound must pass [8]. Another useful analogy is juggling (Figure 2.7). A diverse ensemble of crucial elements must be simultaneously monitored and kept in balance in order to achieve success. Neglecting one element can cause the whole ensemble to crash.

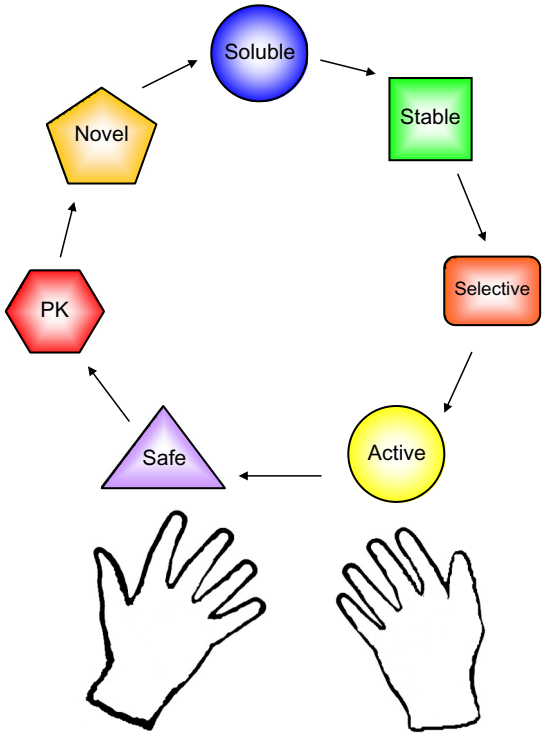

FIGURE 2.7 Success in drug discovery requires simultaneously juggling diverse competing variables.

2.5 PROPERTY PROFILING IN DRUG DISCOVERY

Availability of physicochemical, metabolic, and safety data enables drug discovery scientists to systematically enhance drug properties during drug discovery. Methods have been implemented to provide drug discovery scientists with the necessary information. Data from these methods are provided quickly on a time schedule that is consistent with other investigations in drug discovery. Most organizations strive to obtain reliable quality data for a comprehensive set of properties for newly synthesized compounds. Development colleagues in pharmaceutics, metabolism, toxicology, PK, chemical process, and analytical initially provided assistance in implementing such methods and interpreting data. However, drug discovery applications require distinctly different methods and strategies than development, because of the differences in goals and activities [8]. Drug discovery invents new candidates and development fulfills the requirements for regulatory approval and drug product development.

Current methods for property prediction and measurement are discussed in later chapters. These chapters provide information on the various tools that are available for property assessment. They provide insight on how data are produced by ADME (absorption, distribution, metabolism, and excretion) scientists for project teams. This leads to better interpretation and application of the property data by medicinal chemists. The information also allows medicinal chemists to select among available methods for implementation in their organization.

2.6 DRUG-LIKE PROPERTY OPTIMIZATION IN DRUG DISCOVERY

This book provides resource material for medicinal chemists, drug discovery biologists, students, and development colleagues who are interested how ADME/Tox properties are integrated into their selection and optimization of leads and candidates.

The strategy of SPR complements the traditional strategy of SAR. The structures of compounds are correlated to their property performance. SPR allows medicinal chemists to understand how structural modifications improve properties for their scaffold. The established strategy of structure-based design is thus supplemented with the new strategy of "property-based design" [9], the study and modification of structures to achieve property improvement.

There are many reasons for a drug discovery project team to strive toward selecting leads with good drug-like properties and optimizing properties for their lead chemical series during drug discovery. Property optimization can be approached in balance with activity and selectivity optimization [10,11]. Practical advantages of good drug-like properties include the following:

- Better planning, execution, and interpretation of drug discovery experiments
- Reduced discovery time lag caused by later having to fix property-based problems
- Faster and more economical pharmaceutical development
- Candidates with lower risk and higher future value
- Longer patent life
- Higher patient acceptance and compliance
- Strong due-diligence package for development advancement
- Quality human PK and safety prediction for Phase I

PROBLEMS

(1) How do medicinal chemists change compound properties?
(2) In addition to structure, what determines physicochemical (e.g., solubility) and biochemical properties of a compound?
(3) How can drug-like properties be used in each stage of drug discovery (Figure 2.3)?
(4) How can drug discovery scientists assess properties?
(5) How do poor properties affect drug development, clinical application, and product lifetime?
(6) How do drug properties affect drug discovery biological experiments?
(7) Define and describe SPR.
(8) Which of the following are advantages of optimizing drug-like properties: (a) better quality drug product, (b) lower risk of failure, (c) faster and less expensive drug development, (d) lower cost of goods, (e) more reliable drug discovery biological data, and (f) easier synthesis.

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Chapter 3

In Vivo Environments Affect Drug Exposure

3.1 INTRODUCTION

As soon as a drug is administered to a living system, the drug molecules begin to interact with the physicochemical and biochemical environments that they encounter. Many of these reduce the rate or extent of exposure of the drug molecules to the target, depending on the properties of the drug. A few physiological environments can enhance exposure.

This concept is illustrated in Figure 3.1. The drug molecules can be impeded, which reduces exposure of drug molecules to the target. These challenges comprise many diverse in vivo environments, including lipid bilayer membranes, efflux transporters, metabolic enzymes, and solution pH.

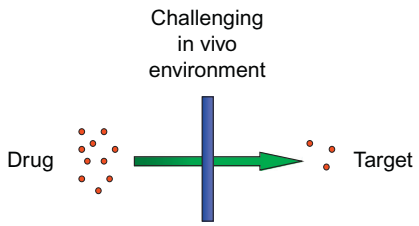


FIGURE 3.1 Model for challenging environments in living systems that reduce drug exposure to the therapeutic target [1]. *(Reprinted with permission of E.H. Kerns, L. Di, Pharmaceutical profiling in drug discovery, Drug Discovery Today 8 (2003) 316–323. Copyright 2003 Elsevier.)*

Drug molecules are exposed to the ensemble of diverse environments. Some are close to the site of dosing (e.g., intestinal epithelial cells), some are between the dose location and the target (e.g., hepatic metabolic enzymes), some are near the target (e.g., efflux transporters in the blood-brain barrier (BBB)), and some are in tissues that are remote from the target. The drug's pharmacokinetics (PK) at the therapeutic target results from the composite behavior of the drug molecules in these challenging environments.

Together these environments determine the PK of the drug at the target and throughout the other tissues. In vivo efficacy is influenced by the PK of target exposure, extent, and time profile of binding (e.g., IC_{50}) to the in vivo target and how that target actually affects the disease [2].

The behavior of a drug molecule in each environment is the direct result of the drug's properties that are determined by its chemical structure (see Chapter 2). In drug discovery, medicinal chemists have the opportunity to modify the structure to optimize performance of a chemical series vis-à-vis the challenging in vivo environments in concert with optimizing activity. This illustrates a Yin & Yang relationship of drugs (Figure 2.6). Achieving a drug that is efficacious in vivo requires activity and properties to be in balance:

(1) Strong target binding (achieved using activity-based design and structure-activity relationship (SAR))
(2) Strong in vivo target exposure (achieved using property-based design [3,4] and structure-property relationship (SPR))

Drug candidates that lack quality in both target binding and target exposure risk disappointing in vivo efficacy.

In this chapter, we will explore the environments in living organisms from the standpoint of the drug molecule. Its behavior at the various organ systems and the physicochemical and biochemical environments it encounters is a fascinating journey. For the purposes of this introduction, it is useful to consider environments in sequence as drug molecules move from oral dosing toward the therapeutic target. With other dosing routes, the drug enters the living system at other places. After dosing, drug molecules disperse throughout the body, encountering and interacting with the diverse environments dynamically. Poor performance in one challenging environment might negate excellent performance elsewhere. For

Drug-Like Properties. http://dx.doi.org/10.1016/B978-0-12-801076-1.00003-4

example, a particular drug might have excellent BBB permeability, but if it has low absorption or high first-pass metabolism, there may not be a high enough plasma drug concentration to get to the brain to produce efficacy.

3.2 DRUG DOSING

First, we consider how and where the drug is administered, which has a great effect on PK. A common goal for the drug product profile, to which drug discovery teams often aspire, includes

- oral administration
- once per day
- solid tablet
- low dose.

Oral administration is abbreviated as PO (*per os*). A drug product of this type has reasonable manufacturing and storage costs and high patient compliance. Clinicians might use other routes for some therapies (e.g., intravenous (IV) for cytotoxic cancer drugs) or the course of treatment or site of action might favor another route (e.g., topical for extended release in skin). If a drug has limited performance at one or more in vivo environments when dosed orally, it might have poor PK performance and will require structure modification or dosing changes. Examples are

- short PK half-life might require more frequent dosing,
- low bioavailability might require higher doses,
- low intestinal absorption might require administration by a different route,
- low solubility might require a different vehicle or formulation

If oral dosing does not produce sufficient exposure, another route of administration, such as IV, is necessary (see Table 3.1 and Section 41.1). However, moving away from oral dosing is likely to limit the patient population that will use the drug product. Non-oral routes are also used during drug discovery, before properties are optimized for good oral absorption. Formulations can improve absorption by increasing the dissolution rate or solubility of the drug (see Chapter 41).

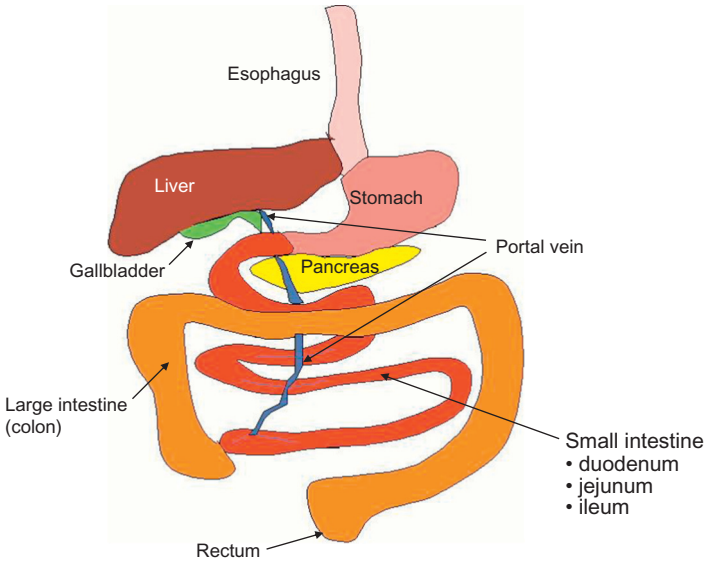
TABLE 3.1 Dosing Routes

Administration	Description	Abbreviation
Oral	Swallowed by mouth or gavage	PO
Intravenous	Injected directly into the vein as a bolus (rapidly) or by infusion (continuously)	IV
Subcutaneous	Injected under the skin	SC
Transdermal	Applied as a patch or other device and absorbed through the skin	TD
Topical	Applied as a solution or suspension on the skin	top
Intramuscular	Injected into the muscle	IM
Epidural	Injected into the epidural space just inside the bone of the lower vertebrae	ED
Rectal	Placed in the rectum	PR
Intranasal	Sprayed into the nose	INS
Buccal	A tablet is held inside the mouth between cheek and gum until dissolved	Buc
Sublingual	A tablet is held underneath the tongue until dissolved	SL
Intraperitoneal	Injected within the peritoneal (abdominal) cavity	IP

3.3 STOMACH

In oral dosing the compound first encounters the mouth, usually briefly. A portion of the drug can be absorbed in the mouth if it stays in the mouth for some time. The buccal and sublingual dosing routes involve keeping the drug in the mouth for an extended time, during which the drug is absorbed through membranes of the mouth into the blood capillaries.

FIGURE 3.2 Diagram of the gastrointestinal tract.



The drug tablet is ingested via the esophagus and arrives at the initial portion of the gastrointestinal (GI) tract, the stomach (Figure 3.2). The drug tablet dissociates into smaller particles, owing to the aqueous environment of the stomach.

For most drugs, absorption from the stomach is limited. This is because the stomach surface area is relatively small (about 1 m^2). In addition, drug material does not stay in the stomach very long. The gastric emptying time is about 0.5 h for fasted state and 1 h for fed state.

3.3.1 Gastric Acidic Degradation

In the stomach, the drug molecules encounter low pH. In the fasted state, the stomach pH is between 1 and 2 and in the fed state it is between pH 3 and 7. Compounds that have *acid instability* might be decomposed by hydrolysis (see Chapter 13).

3.4 INTESTINAL ENVIRONMENT

The stomach contents empty into the duodenum, the first region of the small intestine. Later regions in sequence are termed the jejunum and ileum. The intestinal pH is higher than in the stomach, varying from pH 4.4 in the duodenum in the fasted state to pH 7.4-8 at the end of the ileum. The pHs of the intestinal regions are listed in Table 3.2. This progression of pH creates a pH gradient from the stomach through the small intestine. The transit time is the amount of time available for drugs to be absorbed in that region.

TABLE 3.2 pHs and Transit Times of Regions in the Gastrointestinal Tract of Human

GI Tract Region	Avg. pH—Fasted	Avg. pH—Fed	Transit Time (h)
Stomach	1.4-2.1	3-7	0.5-1
Duodenum	4.4-6.6	5.2-6.2	2-4
Jejunum	4.4-6.6	5.2-6.2	
Ileum	6.8-8	6.8-8	

In the small intestine, drug molecules encounter an anatomy that greatly enhances absorption. The inner surface area of the intestinal lumen is enhanced approximately 400-fold by three morphological features. Along the length of the intestinal lumen there are folds, which are up and down undulations along the inner surface. In addition, villi add to the surface area by projecting 1 mm into the intestinal lumen (Figure 3.3). A layer of epithelial cells covers the surface of the villi (Figure 3.4). Another morphological feature that enhances surface area is the microvilli on the luminal side of the epithelial cells, as shown in Figure 3.5. The microvilli extend about 1 μm into the lumen and are called the brush border.

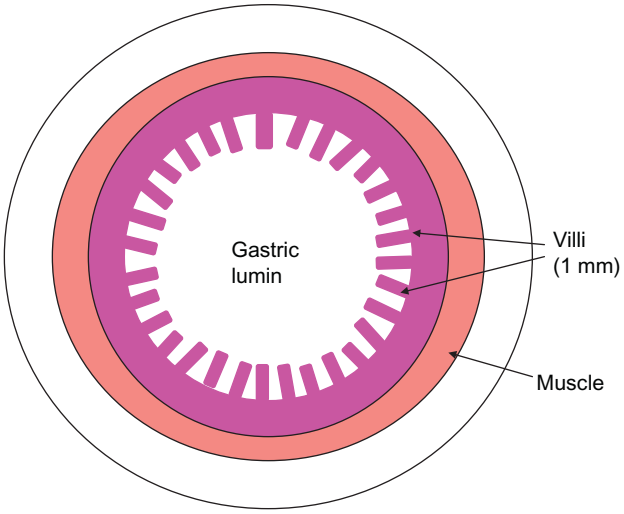


FIGURE 3.3 Diagram of the cross-section of the small intestine.

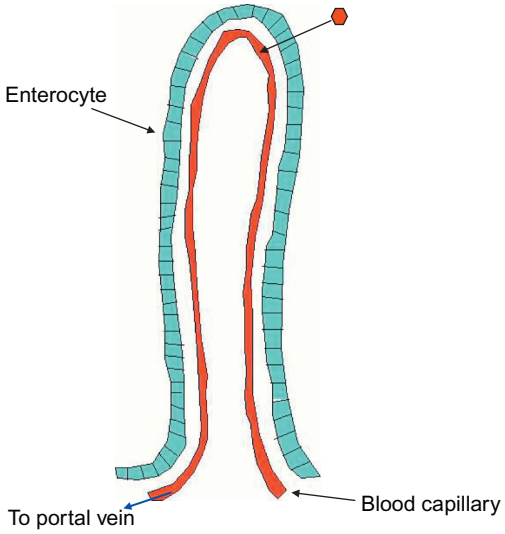


FIGURE 3.4 Functional diagram of a gastrointestinal villus.

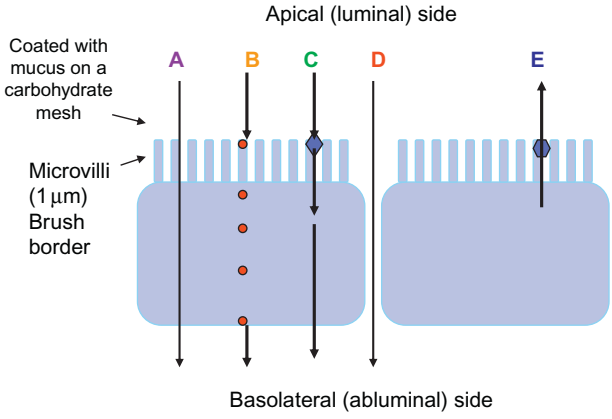


FIGURE 3.5 Permeation mechanisms through the gastrointestinal endothelial cells: (a) passive transcellular diffusion, (b) endocytosis, (c) uptake transport, (d) paracellular, and (e) efflux transport.

Permeation of the layer of epithelial cells is a potential challenge to drug molecule absorption. A drug molecule must pass through this cellular membrane to reach the blood capillary and subsequent systemic circulation.

The low pH of the upper small intestine continues to enable hydrolysis. Some structures are susceptible to acidic hydrolysis.

3.4.1 Dissolution Rate

Dissolution rate is the transfer rate of individual drug molecules from the solid particles (usually crystalline) into solution as individual free drug molecules. Dissolution rate is determined by the crystal forces. Molecules must be free in solution to permeate across the intestinal cell membrane for absorption to occur. Factors that affect dissolution rate are discussed in Chapter 7. Dissolution rate can be improved by reducing particle size (e.g., grinding the solid drug active ingredient or forming smaller particles), which increases surface area per unit of mass. With greater surface area, more of the compound is solubilized in the same time. Salt form can also be manipulated to increase dissolution rate. Several possible counter ions are often screened to select a salt form having a higher dissolution rate. Formulation is also manipulated to enhance dissolution rate. Embedding the compound in excipients that break apart in an aqueous environment can rapidly disperse the compound particles or molecules in the stomach and upper intestine, thus increasing the dissolution rate (see Chapter 41).

3.4.2 Solubility

Higher *solubility* produces a greater concentration of free drug molecules at the intestinal membrane for absorption. As the concentration gradient across the membrane increases, the flux of drug molecules across the membrane also increases. Solubility varies throughout the length of the intestine, because it is greatly affected by the solution pH and the pK_a of the molecule (see Chapter 7). Most basic molecules are in the charged cationic (protonated) state throughout the stomach and intestine. This favors good solubility, because the charged form is more soluble than the neutral form. Most acid molecules are neutral in the stomach and upper intestine, thus limiting solubility to the intrinsic solubility of the neutral molecules. As the pH increases throughout the intestine, the relative amount of the anionic form of the acid increases, resulting in higher solubility. These behaviors are examples of the solubility differences among compounds in different regions of the intestine. The fundamentals and effects of pK_a on solubility are discussed in Chapter 6. Solubility can be enhanced with structural modifications that introduce a solubilizing functional group, such as one that is ionizable (see Chapter 7).

3.4.3 Permeability

Permeability, if it is low, can impede absorption in the intestine (see Chapter 8). As with solubility, permeability varies with the pH of the intestinal region and the compound's pK_a. Molecules in their neutral form have much greater permeability than their charged form. (Conversely, neutral molecules are less soluble than their charged form. Thus, permeability and solubility vary inversely with pH.) Molecules permeate through cellular membranes by several different mechanisms, as shown in Figure 3.5.

Passive transcellular diffusion is the predominant permeation mechanism for most drugs (see Chapter 8). The lipid bilayer membrane is represented in Figure 3.6. It consists of phospholipid molecules that self-assemble as a bilayer, with

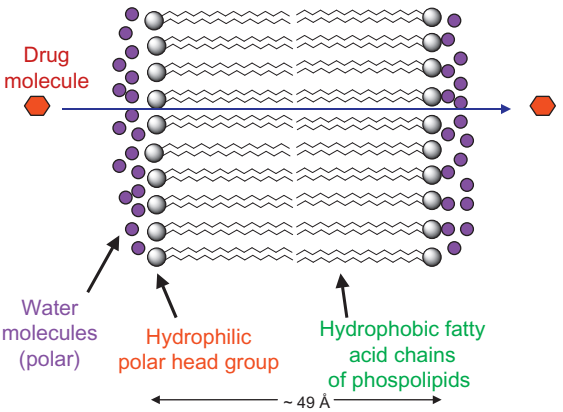


FIGURE 3.6 Passive diffusion of drug molecule through lipid bilayer membrane.

the aliphatic portion on the inside, away from the polar water molecules, and the polar phosphate and hydrophilic head groups oriented toward the water molecules. Passive lipoidal diffusion involves movement of drug molecules through the lipid bilayer as follows. The hydrating water molecules around the drug molecule are shed and hydrogen bonds are broken. The molecule then passes through the region of polar head groups of the phospholipid molecules. It then encounters the tightly packed lipid chains of the bilayer. Larger molecules (higher molecular weight, MW) do not pass through the tightly packed region as readily as smaller molecules [5]. Molecules with higher lipophilicity are typically more permeable than less lipophilic molecules through the highly nonpolar central core of the lipid bilayer membrane. Molecules then move through the side chains and polar head groups of the inner portion of the bilayer and are rehydrated by water molecules at the other side of the bilayer.

The chemical structures of representative phospholipid molecules are shown in Figure 3.7. One of the alcohol groups of the glycerol backbone is attached to a phosphate group, which is attached to a head group. Examples of head groups of common phospholipids are shown in Figure 3.7. Phosphatidylcholine is a common phospholipid found in many membranes. The head groups impart a charge and polarity to the outside of the membrane. Membranes also contain other components, such as cholesterol and transmembrane proteins (e.g., channels, transporters, receptors). The membranes in a specific tissue are composed of a specific mixture of phospholipids and other components, which may differ from other tissues. A compound might have different passive diffusion membrane permeability in different tissues of different lipid composition (e.g., GI tract vs. BBB).

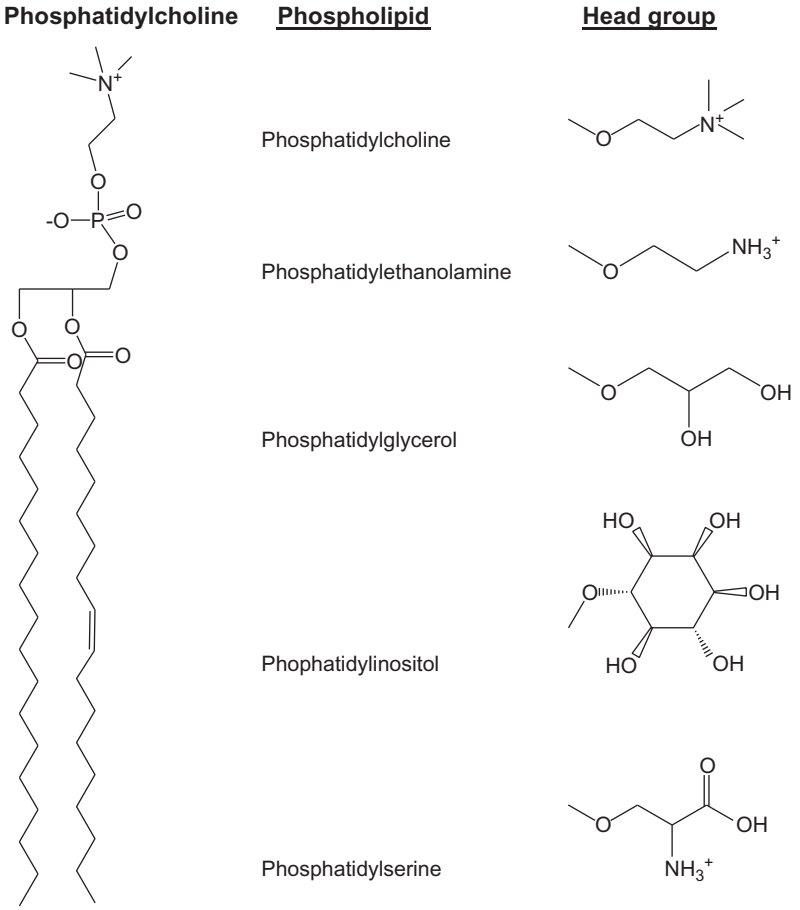


FIGURE 3.7 Structures of some common phospholipids.

Uptake transport is performed by transporters in the membrane for drug molecules that are ligands (see Chapter 9). These enhance the membrane permeation of compounds (e.g., nutrients, drugs) that are too polar to permeate via passive transcellular diffusion.

Efflux transport is performed by other membrane transporters. These reduce permeation of drug molecules that are ligands. P-glycoprotein (P-gp) is a well-known efflux transporter.

Paracellular permeation occurs when drug molecules permeate the cell membrane by moving through the tight junctions of the cells. In the intestine, paracellular permeation occurs for small polar compounds.

Endocytosis occurs when extracellular molecules are engulfed along with a small volume of extracellular solution by the membrane and move through the cell in vesicles that are released on the other side of the cell. This enhances permeation for some compounds, but is not a significant route for small drug molecules.

3.4.4 Intestinal Metabolism

Metabolism occurs for some compounds in the GI. Cytochrome P450 3A4 isozyme (CYP3A4) is the most abundant metabolic enzyme in intestinal epithelial cells. This enzyme metabolizes diverse compound structures. Intestinal metabolism is considered part of "first-pass metabolism," which is the initial metabolism of drug molecules in the intestine and liver before they reach the systemic circulation. CYP3A4 has similar substrate specificity to P-gp and they functionally work in concert. P-gp reduces the intracellular drug concentration in enterocytes and this level is more efficient for CYP3A4 metabolism [2]. Lower levels of other CYP enzymes and Phase II metabolizing enzymes (e.g., UDP-glucuronosyltransferases (UGTs) that catalyze glucuronide conjugate formation) also occur in enterocytes.

3.4.5 Intestinal Enzymatic Hydrolysis

Hydrolytic enzymes are present in the intestinal lumen. They can catalyze the hydrolysis of some drug molecules that contain hydrolysable functional groups (see Chapter 13).

The natural function of the GI system is the digestion and absorption of nutrients to sustain the living system. Food contains macromolecules that are made up of the monomers that are needed to produce energy and build specific proteins, carbohydrates, and nucleic acids for that organism. Pancreatic fluid is added to the material exiting the stomach and it contains hydrolytic enzymes. It contains amylases, lipases, and proteases. Other enzymes are secreted by the salivary glands and stomach. The enzymes break down macromolecules in food. Protein digestion to peptides and amino acids is accomplished by peptidases, such as pepsin, that are secreted into the stomach, and trypsin and chymotrypsin that are secreted by the pancreas into the small intestine. Fat digestion to fatty acids is performed by esterases, such as lipase, that is secreted by the pancreas into the small intestine. Ribonuclease and deoxyribonuclease digest RNA and DNA, respectively. Phosphatases and phosphodiesterases are other common enzymes.

GI enzymes can also catalyze drug hydrolysis. Drugs that contain derivatives of carboxylic acids such as esters, amides, and carbamates are especially susceptible. Enzymes are found in the intestinal lumen especially at the brush border. Thus, drugs can be hydrolyzed before they reach the bilayer membrane (see Chapter 13). Usually, it is not known which enzyme is decomposing the drug molecules when intestinal hydrolysis is observed.

Prodrugs are designed to take advantage of hydrolysis (see Chapter 39). Compounds that have a desirable pharmacological effect, but lack sufficient solubility for absorption, have been modified to add a substructure that increases solubility in the intestine (e.g., phosphate). The increased solubility allows the modified compound to diffuse through the lumen to the epithelial cell surface. A hydrolytic enzyme (e.g., phosphatase) then cleaves off this substructure in the vicinity of the bilayer membrane. The active drug is released and permeates through the epithelial cells to reach systemic circulation.

The challenges to drug absorption in the GI tract are summarized in Figure 3.8 and Table 3.3. The dynamic balance of permeation mechanisms (passive, active uptake, and efflux), pH and enzyme-induced hydrolysis, CYP metabolism,

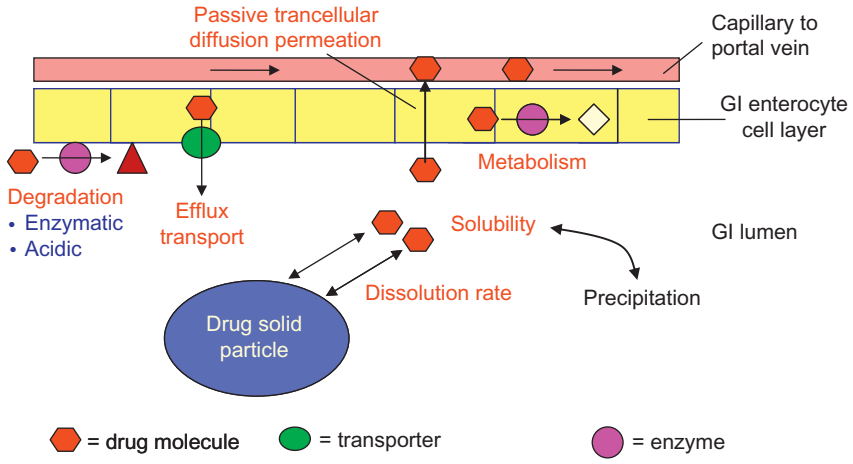


FIGURE 3.8 Composite diagram of features of the intestine that challenge drug absorption.

TABLE 3.3 Drug Challenges in the Gastrointestinal Tract

Property	Description
Dissolution rate	Rate of transfer of compound from the surface of the particle to aqueous solution
Solubility	Maximum concentration that can be reached under the present conditions
Permeability	Movement from an aqueous solution through a lipid membrane to the aqueous solution on the other side
Chemical instability	Reaction of compound as a result of an environmental condition (e.g., pH, light, heat, oxygen, water)
Hydrolyzing enzymes	Naturally occurring enzymes that catalyze hydrolysis of endogenous and food molecules and can catalyze hydrolysis of some drugs

solubility and dissolution rate affect the net rate of absorption in the intestine. It is useful for medicinal chemists to consider all of these mechanisms in trying to diagnose the causes of poor PK (see Chapter 38).

3.4.6 Absorption Enhancement in the Intestine

There are some factors that can enhance absorption of certain drugs in the intestine (Figure 3.9). Enhancement by the high intestinal surface area is discussed in Section 3.4.

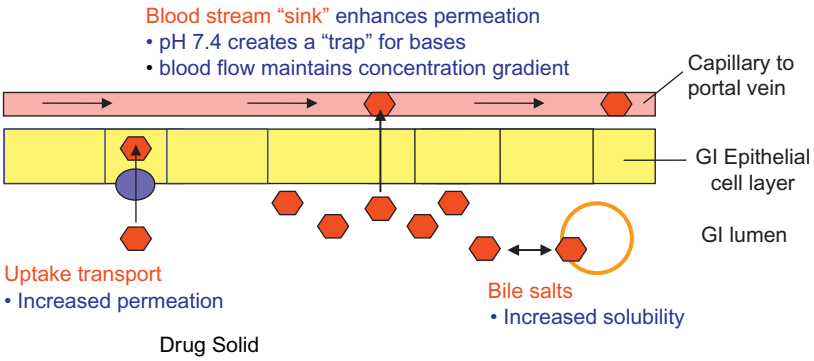


FIGURE 3.9 Gastrointestinal tract features that enhance drug absorption.

The material coming from the stomach is mixed with bile from the gallbladder in the intestine. Bile salts (e.g., taurocholate, glycocholate) enhance the solubility of lipophilic drug molecules, by forming micelles that adsorb lipophilic molecules and enable them to circulate in solution away from the solid crystals. When the drug molecules reversibly release from the micelles near the intestinal membrane they have access to permeate. The natural function of bile acids is to solubilize food lipids to enhance absorption. Food intake stimulates the release of bile salts.

Uptake transporters can provide enhancement of absorption of certain drugs. The natural function of uptake transporters is to enhance nutrient absorption. If the molecule has affinity for a transporter, its absorption might be enhanced (see Chapter 9).

Absorption is increased by a "sink" effect, wherein the flowing blood in the capillary system sweeps away drug molecules that have permeated through the enterocytes. Drug molecules move into the portal vein and quickly away from the intestine. This maintains a high drug concentration gradient across the enterocytes that drives passive transcellular diffusion in the absorptive direction.

3.5 BLOODSTREAM

Once drug molecules reach the bloodstream, they encounter new environments. Each of these can reduce the drug concentration in systemic circulation, thus reducing penetration into the tissues.

3.5.1 Plasma Enzyme Hydrolysis

Enzymatic hydrolysis can occur in plasma for certain drugs. A large number of enzymes are present in blood for natural functions, but can also catalyze drug decomposition. These include cholinesterase, aldolase, lipase, dehydropeptidase, alkaline and acid phosphatase, glucuronidase, dehydrogenase, and phenol sulfatase. The substrate specificity and relative amount of these enzymes vary with species, disease state, gender, age, and race. They differ from the enzymes in the GI tract. The most common reaction is hydrolysis (see Chapter 12).

3.5.2 Plasma Protein Binding

Plasma protein binding (PPB) occurs when drug molecules bind to plasma proteins (see Figure 3.10). Approximately 6-8% of plasma is protein and a large percentage of this serves as a carrier for natural in vivo compounds. Drug molecules often reversibly bind to these proteins. This is termed PPB. The affinity of binding is determined by the structural properties of the drug. PPB determines the ratio of bound and free drug in plasma. There is high capacity for drug binding in plasma and it is normally not saturated unless the drug concentration is very high. Protein binding results in a constant fraction of bound versus free drug molecules, over a wide total drug concentration range. The concentrations of plasma proteins can vary with disease state and age. There are two major types of drug binding proteins in plasma: albumin and α_1-acid glycoprotein.

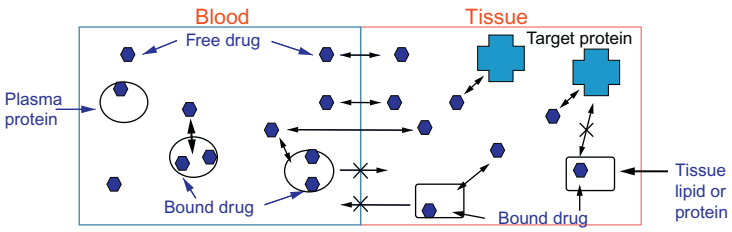


FIGURE 3.10 A fraction of the drug molecules in blood bind to albumin or/and α1-acid glycoprotein. Only the free (unbound) molecules enter the intracellular fluid by passing through the cell membranes to reach intercellular target proteins. In tissue, drug molecules bind nonspecifically to lipids and proteins. Only the free molecules in tissue bind to the target proteins.

Human serum albumin (HSA) has at least six binding sites that have broad ligand binding specificity. Two sites bind fatty acids and another binds bilirubin. Two sites bind acidic drugs. Warfarin and phenylbutazone bind to one site, and diazepam and ibuprofen to another [6]. Other drugs can also bind to sites on HSA.

Basic drugs can bind to α_1-acid glycoprotein. This protein has up to seven binding sites. Examples of drugs that bind to α_1-acid glycoprotein include disopyramide and lignocaine. This protein can be saturated at higher drug concentrations.

Protein binding has several effects on drug disposition, which can have complex and counteracting effects. Examples of the effects of PPB are as follows:

- Only free drug molecules move through membranes from the blood in the capillaries to the intracellular fluid in the tissues. Therapeutic efficacy requires the drug to reach a certain free concentration in the biophase around the target. The free drug concentration in tissue usually reaches equilibrium with the free drug concentration in plasma, unless the rate or extent of penetration into the tissue is reduced by other factors.
- Only free drug molecules permeate into the liver and kidney for clearance.

The overall effects of PPB are complicated and easily confused. A discussion of PPB is in Chapter 14.

3.5.3 Red Blood Cell Binding

Drug molecules can bind to red blood cells. This is primarily a lipophilic interaction with the cell membrane. Drug discovery projects often check for red cell partitioning (blood to plasma ratio) of lead compounds.

3.6 LIVER

Drug molecules can be affected by multiple factors in the liver. The liver is one of the two major organs of drug clearance from the body. A functional diagram of the liver is in Figure 3.11.

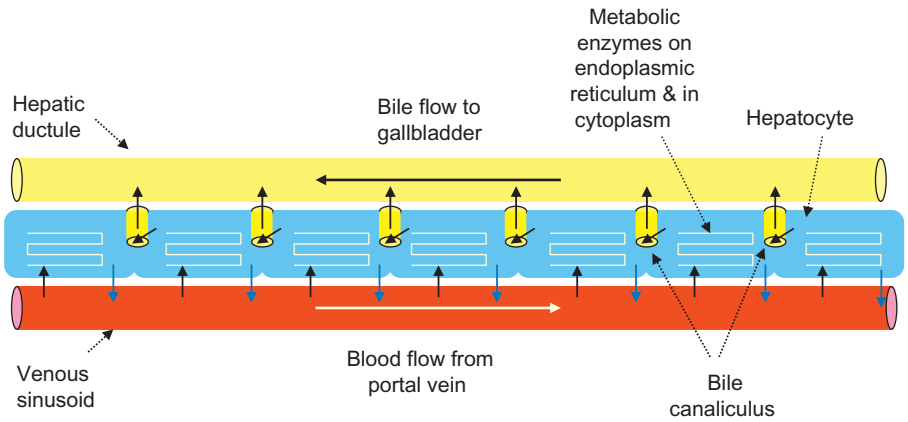


FIGURE 3.11 Functional diagram of hepatic clearance in hepatocytes by metabolism and extraction into bile.

3.6.1 Permeation into and out of Hepatocytes

Within the liver, the portal vein that carries absorbed drug molecules from the intestine branches into successively smaller capillaries. The narrowest are called venous sinusoids. They are in close proximity to hepatocytes, which form cell sheets that, in the diagram, are arrayed perpendicular to the page. Drug molecules move out of the venous sinusoids into the fluid that surrounds the hepatocytes and then permeate into the hepatocytes. The permeation into hepatocytes occurs via passive lipoidal diffusion and active uptake transport mechanisms as discussed in Section 3.4.4). The rate of permeation into hepatocytes can affect the PK of the drug. Drug molecules also move from the hepatocytes to blood via passive diffusion and efflux transporters.

3.6.2 Hepatic Metabolism

A diverse array of metabolizing enzymes are present within hepatocytes, with a wide specificity for binding and catalyzing metabolic reactions to drug molecules. *Hepatic metabolism* occurs via two types of metabolic reactions. The first, Phase I, causes chemical changes to the drug molecule (e.g., hydroxylation), many of which are oxidative. The second type of reaction is Phase II, which adds polar molecules to the drug molecule. Metabolism serves the natural function of making xenobiotic compounds more polar so that they have higher water solubility to be eliminated through the kidney (see below). Metabolism might also reduce the toxicity of xenobiotic compounds, but toxic metabolites can be produced. A high rate of liver metabolism results in (a) high first-pass effect (i.e., metabolism prior to reaching systemic circulation), (b) high clearance, (c) reduced exposure, and (d) low bioavailability. Metabolism is a major route of drug clearance (see Chapter 11).

3.6.3 Biliary Extraction

Drug molecules and metabolites can permeate out of hepatocytes into the bile canaliculus, a capillary duct that forms at the junctions between hepatocytes. This permeation might be via passive lipoidal diffusion or efflux transporters on the canalicular membrane. For example, P-gp and MRP2 are active efflux transporters at the canalicular membrane. The natural function of the bile canaliculus is to collect secretions from hepatocytes to form bile and eliminate xenobiotics. Bile moves into the hepatic ductule and then into the gallbladder. Bile is released from the gallbladder into the small intestine and, for certain compounds, results in excretion of a significant amount of drug and metabolites in the feces. Drug molecules and metabolites might be reabsorbed, but metabolites are less readily absorbed because they are more polar, unless they are converted back to the parent molecule (enterohepatic circulation). The fraction of drug excreted by this route depends on the properties of the compound.

3.7 KIDNEY

Renal excretion occurs via the kidney. A functional diagram of the kidney nephron is in Figure 3.12. The product of the kidney is urine, in which polar drug molecules and some metabolites are excreted. The permeation mechanisms are passive diffusion, paracellular, uptake transport, and efflux transport.

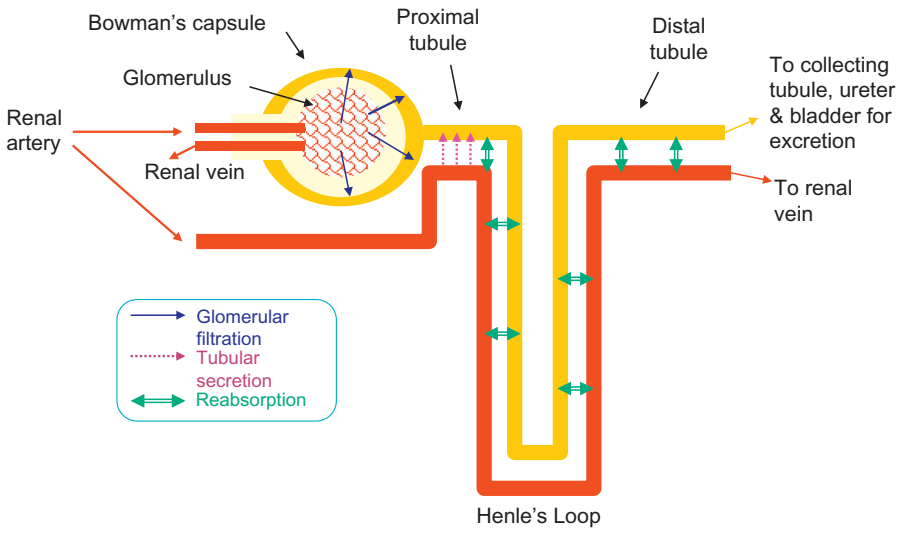


FIGURE 3.12 Diagram of drug excretion via the kidney nephrons.

There are about a million nephrons in a kidney. Blood flow from the renal artery divides into capillaries of the nephrons, part going to glomerulus and part going to a capillary that is in close proximity to the nephron tubules. The first stage is termed "glomerular filtration." As blood passes through the glomerulus (1200 mL/min in human), about 10% is filtered as plasma water into the renal tubule (120 mL/min = glomerular filtration rate or GFR for human). The glomerulus is a complex network of blood capillaries and it presents a high surface area to the Bowman's Capsule, which is connected to the tubules. The leaky pores of the glomerulus allow lower molecular weight blood components (e.g., electrolytes, drug molecules, metabolites) to be filtered with water, but not normally proteins or cells, to the Bowman's Capsule.

This fluid then moves into the proximal tubule. On the cells forming the proximal tubule there are transporters that move drug molecules and metabolites from the adjacent blood capillary into fluid in the proximal tubule. This is termed "tubular secretion". The transporters include organic anion transporters (OATs for penicillins and glucuronide metabolites), organic cation transporters (OCTs for morphine and procaine), P-gp (for digoxin), MRP2, and MRP4 [6]. Passive diffusion also occurs in both directions, but it appears to have an approximately net zero effect on excretion. The net result of tubular secretion is that ligands for the transporters on the proximal tubule cells are excreted.

Next, much of the water (99%) is reabsorbed into the blood. Water reabsorption increases the concentration of the remaining solutes in solution in the renal tubule. In the distal tubule, these concentrated solutes (e.g., drugs, valuable biochemicals) can permeate by passive diffusion from the fluid back into the blood. The permeation of a particular drug is dependent on its physicochemical properties (e.g., lipophilicity, TPSA). The net result of reabsorption is that more lipophilic drug molecules tend to permeate back into the blood and be reabsorbed, because they have higher permeability by passive diffusion. The more hydrophilic drug molecules tend to stay in the aqueous fluid in the tubules and are excreted in the urine. Metabolites in the blood, being more polar than their parent drug molecules, can be excreted in this manner.

Hydrophilic drug molecules tend to be cleared unchanged via the kidney into the urine. Conversely, lipophilic drug molecules tend to be cleared by metabolism (their metabolites being eliminated via the feces or urine).

3.8 BLOOD-TISSUE BARRIERS

Blood-tissue barriers are found on highly sensitive organs and they reduce the penetration of certain drugs into the organ tissue. Such barriers exist at the placenta, testes, and brain. The BBB is the best-known barrier. This barrier is formed by the endothelial cells of the capillary blood vessels that perfuse the brain. The endothelial cells reduce penetration of the drug molecules by multiple mechanisms. One mechanism is lack of fenestrations and impenetrable tight junctions between membrane cells that do not permit paracellular permeation. Another mechanism is high expression of efflux transporters that actively remove drug molecules from inside the cells or the membrane. A major frustration in discovering drugs for central nervous system (CNS) disorders is penetration of the BBB (see Chapter 10).

3.9 TISSUE DISTRIBUTION

The bloodstream carries drug molecules throughout all the tissues of the body. Distribution of drug into nontarget tissues serves as a depot of the drug that affects PK volume of distribution and PK half-life.

Certain drugs preferentially depot in certain tissues. For example, lipophilic compounds tend to accumulate in adipose tissues. Acidic compounds accumulate in muscles, which has a pH of approximately 6. The pH's of various physiological fluids and organs are shown in Table 3.4.

TABLE 3.4 pHs of Physiological Fluids of Humans

Physiological Fluid	pH
Blood	7.4
Stomach	1-3
Small intestine	5.5-7
Saliva	6.4
Cerebral spinal fluid	7.4
Muscle	6
Urine	5.8

Blood flow to an organ affects the time taken for the organ tissue drug concentration to equilibrate with the blood. The high cardiac output (blood flow) to heart, lungs, liver, kidney, and brain allows rapid equilibration of drugs with those organs. Cardiac output is lower to skin, bone, and fat resulting in slower equilibration in those tissues.

3.9.1 Nonspecific Binding in Tissue

Tissue binding occurs when drug molecules permeate into tissue and bind nonspecifically to lipids and proteins in the tissue (Figure 3.10). In tissue at equilibrium, a fraction of molecules are bound and a fraction are free. The free drug fraction in a tissue is usually different than that in plasma. The important point for efficacy is that only the free drug molecules bind to the therapeutic target in the target tissue to produce the therapeutic effect. The consequence is that the free drug concentration in the biophase surrounding the target determines the efficacy.

3.10 CONSEQUENCES OF CHIRALITY

Chirality can have a significant effect on the behavior of compounds in vivo. It affects many properties, owing to the chiral interaction of different enantiomers with proteins. This affects the compound's PK. Examples of properties affected by chirality and the causes (in parentheses) are

- dissolution rate (crystal forms of enantiomers may be different)
- efflux and uptake transport (different binding to transporter)
- metabolism (binding and orientation of the drug molecule's reactive moiety to the active site on the enzyme are different)
- PPB (binding to a specific site)
- toxicity, such as CYP inhibition or hERG blocking (binding).

An example is shown in Table 3.5. These drugs have differences in renal clearances owing to chirality. This is likely caused by differences in active transport in the nephrons (e.g., active secretion) or by PPB. Additional discussion on the effects of chirality is found in chapters on specific properties.

TABLE 3.5 Stereoselectivity of Renal Clearance

Drug	Renal Clearance Enantiomeric Ratio*
Quinidine	4.0
Disopyramide	1.8
Terbutaline	1.8
Chloroquine	1.6
Pindolol	1.2
Metoprolol	1.1

**Renal clearance of one enantiomer/renal clearance of other enantiomer.*

3.11 OVERVIEW OF IN VIVO CHALLENGES TO DRUG EXPOSURE

In the following chapters, the effects of individual physiological environments on in vivo delivery of compound to the therapeutic target are discussed in greater detail. Poor delivery of the compound to the target results in reduced target exposure. In vivo environments are summarized in Figure 3.13. It is important for drug discovery project teams to improve how their lead compounds behave in in vivo environments. This is accomplished by assaying the compounds in vitro for key properties that predict performance in these environments and then making structural modifications to improve these properties.

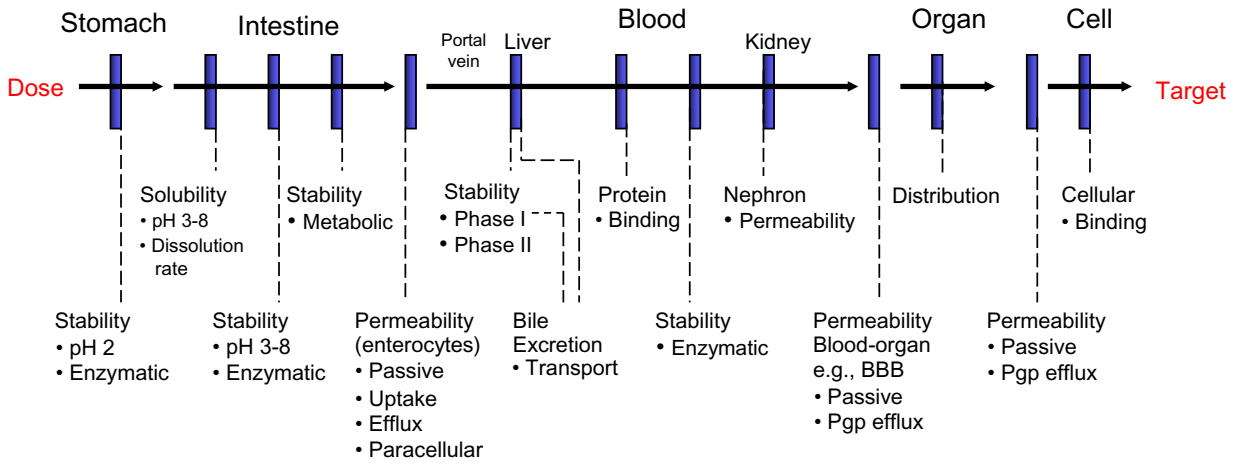


FIGURE 3.13 Overview of in vivo challenges of drug delivery to the target [1]. *(Reprinted with permission of E.H. Kerns, L. Di, Pharmaceutical profiling in drug discovery, Drug Discovery Today 8 (2003) 316–323. Copyright 2003 Elsevier.)*

There is often a trade-off between structural features that enhance therapeutic target binding and structural features that enhance delivery to the target through optimal in vivo performance. If the sole focus of a drug discovery program is on activity optimization, poor properties can result, leading to

- low absorption, owing to low solubility or permeability
- high clearance, owing to metabolism
- clearance, owing to hydrolysis in the GI tract or blood
- efflux that opposes exposure in many organs and enhances extraction in the liver and kidney
- poor penetration of a blood-organ barrier at the target organ.

PROBLEMS

(1) List two factors that affect drug efficacy in vivo.
(2) What is the preferred drug dosage form and regimen?

(3) List some physicochemical and metabolic property limitations that reduce drug target exposure in vivo.
(4) What is the relationship of solubility to absorption?
(5) What is the relationship of permeability to absorption?
(6) What factors make drugs have lower absorption in the stomach than in the small intestine?
(7) Is the pH higher or lower in the fasted state than in the fed state in the stomach?
(8) A greater portion of molecules of a basic compound are neutral in the (a) upper intestine or (b) lower intestine. A greater portion of molecules of an acidic compound are ionized in the (a) upper intestine or (b) lower intestine.
(9) What is mixed with stomach contents as it enters the intestine and what are the effects on drugs?
(10) Charged versus neutral molecules are (a) more permeable, (b) less permeable, (c) more soluble, or (d) less soluble.
(11) Passive diffusion across lipid bilayer membranes is generally higher for molecules with (a) lower lipophilicity and (b) higher lipophilicity.
(12) List three factors that can reduce free drug concentration in the blood stream.
(13) For most drugs, the organs primarily involved in elimination are the (a) stomach, (b) large intestine, (c) portal vein, (d) small intestine, (e) liver, and (f) kidney.
(14) List two clearance mechanisms in the liver.
(15) What barrier limits drug penetration to brain tissue?
(16) Why are metabolites that are circulating in the blood generally more readily extracted by the kidney than the drug from which they were formed?
(17) For most drugs, absorption occurs primarily in the (a) stomach, (b) large intestine, (c) portal vein, (d) small intestine, (e) liver, and (f) kidney.
(18) Total absorption from the intestinal lumen into the blood stream can be affected by which of the following properties of the compound: (a) solubility, (b) permeability, (c) pK_a, (d) P-gp efflux, (e) metabolic stability, (f) molecular size, (g) enzymatic hydrolysis, or (h) blood-brain barrier permeation.
(19) Which of the following can be improved by structural modification of the lead compound: (a) Phase I metabolism, (b) efflux, (c) enzymatic decomposition, (d) solubility, or (e) passive diffusion permeability.

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Chapter 4

Prediction Rules for Rapid Property Profiling from Structure

4.1 INTRODUCTION

The concept of prediction rules started with the "rule of 5" that was published in 1997 [1]. This simple set of structural properties encompasses 90% of new chemical entities (NCEs) that have adequate human pharmacokinetics (PK) and safety after oral dosing and intestinal absorption. The rule of 5 came out amid a movement to expand drug discovery's mandate from finding patentable active therapeutic target ligands to include the mandate of quality human PK and safety.

This enhanced mandate naturally generated concerns among drug discovery scientists that it limits chemical space for drug innovation and that more time is required to discover new clinical candidates. Widespread adoption of the rule of 5 took some time, as shown by continuing increase of log *P* and molecular weight (MW) of advanced discovery leads during the decade after the rule of 5 was published [2]. However, adoption of the rule of 5 was supported by reports of the high attrition rates of compounds, due to PK and toxicity in development, as well as in discovery. Development attrition seems to be eliminating many non-drug-like candidates and collapsing the mean of new commercial oral drugs down to *C*Log*P* $\approx$ 2.4 and MW $\approx$ 340 [3], which is well within the rule ranges. Productivity is improved when control of molecular properties that produce quality PK and safety is integrated into discovery.

In recent years, a new limitation in chemical space has emerged. Molecular properties such as lipophilicity were recognized as a cause of promiscuous toxicity (a.k.a., secondary pharmacology) [4]. This gives even more reason to control structural properties.

Key elements of the rule of 5 are that it is related to the structure, constructed from the everyday tools and language of chemists (lipophilicity, hydrogen bonds, and MW) and is based on solid evidence. Rules link the discovery laboratory to the requirements of New Drug Application (NDA) approvals. They focus discovery work and creativity along pathways of higher success potential. Rules embody the idea of "opportunity cost": when one pathway is chosen, we forgo opportunities of alternative pathways. Having the best information for choosing among pathways gives us the best chances of success.

Prediction rules support these strategies of drug discovery:

(1) Assess early whether compounds fit the drug-like chemical space.
(2) Modify the structure of an intriguing lead to obtain drug-likeness.

The success of the rule of 5 prompted further investigation of molecular property ranges associated with successful leads and clinical candidates. Other rule sets have emerged for important aspects of drug discovery, such as lead-like compounds, good in vivo PK, screening and fragment libraries, blood-brain barrier, and promiscuous toxicity. They provide new insights for discovery scientists.

Drug discovery groups will likely find ways to deliver drug molecules to the therapeutic target that are outside the ranges of the rules for oral absorption and these will provide exciting new opportunities for drug design. However, rules will still pertain to a large portion of drug discovery projects.

4.2 GENERAL CONCEPTS FOR PREDICTION RULES

The development of different rule sets has several commonalities. The developers start with a compound set that has drug-like property measurements from advanced studies, such as human PK after oral dosing or in vivo PK studies with animal species. Multiple molecular properties of the compounds in the set are determined by counting (e.g., H-bond donors (HBD), MW) or computation (e.g., *C*Log*P*, polar surface area (PSA)). These properties are evaluated by various processes (e.g., statistics, multivariate analysis) for their correlation to the advanced study measurements. The properties that have

Drug-Like Properties. http://dx.doi.org/10.1016/B978-0-12-801076-1.00004-6

the greatest correlation to the advanced measurement are evaluated for the value at which a large fraction of the compound set was within the value.

4.3 RULE OF 5

The "rule of 5" [1], a.k.a. Lipinski Rules, is clearly a foundation block of modern medicinal chemistry. It was the first major set of rules and it remains the most important. Medicinal chemists had recognized for years that lipophilicity, hydrogen bonding, and MW were important for drugs, but Lipinski and colleagues undertook a systematic quantitative study. They wanted to set an "absorption-permeability alert procedure to guide medicinal chemists" [1]. Their compound set consisted of 2245 compounds selected as having a World Drug Index (WDI) or United States Adopted Name (USAN) (an indicator that successful human clinical studies have been completed) and not containing a polymer, peptide, quaternary salt, or O=P-O fragment. The WDI and USAN names are assigned between Phase I and II, thus, named compounds had been selected "by economics" by their sponsors as having the drug-like property assessment of sufficient human PK for resources to be invested in Phase II testing. Compounds with major absorption limitations would not have made it to Phase II and not been assigned a name. Based on medicinal chemistry experience and literature, lipophilicity, HBD, H-bond acceptors (HBA), and MW were selected as indicator parameters that were well known to and accepted by medicinal chemists. For rule of 5 purposes, HBD are calculated as: (number O-Hs + number N-Hs) and HBA are calculated as: (number Ns + number Os). The rule of 5 values are each set at the 90th percentile of the compound set, so that chemists are alerted when any of these parameters for their compound are in the range that is statistically less likely to have good absorption or permeation. The rule of 5 is restated as given below:

Compounds are more likely to have poor absorption or permeation if they have:

- H-bond donors > 5
- MW > 500
- *C*Log*P* > 5 (or *M* log *P* > 4.15)
- H-bond acceptors > 10

(Substrates for biological transporters are exceptions.)

Exceeding one of the parameters puts the compound into only 10% of the successful compound set. Exceeding more than one parameter further increases risk. For example, only 1% of the compound set had both MW and log *P* values in excess of the upper ranges.

The rules were used at Pfizer for a few years prior to publication and have since become widely used. The impact of these rules in the field has been high. This acceptance can be attributed to many factors:

- Easy, fast, and no cost to use
- The "5" mnemonic makes them easy to remember
- Intuitively evident to medicinal chemists
- Widely used standard benchmark
- Based on solid research, documentation, and rationale
- Works effectively

Some chemists are initially surprised that every possible hydrogen-bonding atom is not included and that the H-bond strength is not considered. However, in general, these details average out and the rules work well enough for their intended purpose if the hydrogen bonds are added up as stated. The rule of 5 authors intended for the structure to be the central focus and to have a rapid tool. Examples of counting HBD and HBA are shown in Table 4.1. For example, an R-OH counts as both one HBD and one HBA.

There are good physicochemical rationales for the rules. Hydrogen bonds increase aqueous solubility and must be broken for the compound to partition into the lipid bilayer membrane. Thus, an increasing number of hydrogen bonds reduces passive diffusion through the bilayer membrane. MW is related to the size of the molecule. Increasing size impedes passive diffusion through the tightly packed lipid bilayer membrane. Size also reduces solubility, thus reducing the compound concentration at the surface of the intestinal epithelium for absorption. Increasing log *P* also decreases aqueous solubility, which reduces absorption. Finally, membrane transporters can either enhance or reduce compound absorption by either uptake or efflux transport, respectively. Thus, transporters can have a strong impact on increasing or decreasing

TABLE 4.1 Examples of Counting Hydrogen Bonds for the Rule of 5

Functional Group	H-bond Donors	H-bond Acceptors
Hydroxyl	1 (OH)	1 (O)
Carboxylic acid	1 (OH)	2 (2 Os)
Primary amine	2 (NH_2)	1 (N)
Secondary amine	1 (NH)	1 (N)
Aldehyde	0	1 (O)
Ester	0	2 (O)
Pyridine	0	1 (N)

absorption. Compounds that lie outside the rule of 5 tend to be antibiotics, antifungals, vitamins, and cardiac glycosides, which are likely transporter substrates.

Lipinski et al. also discussed important implications of these rules in light of current drug discovery strategies. Lead optimization often increases target binding by adding lipophilicity, thus reducing solubility. Combinatorial chemistry and parallel array synthesis tend to be more facile with more lipophilic groups, thus, analogs tend to have higher lipophilicity. In biology, high-throughput screening (HTS) tends to favor more lipophilic compounds than screening strategies in previous decades, because compounds are first dissolved in dimethyl sulfoxide (DMSO) and not in aqueous media, as in the past. Therefore, to obtain favorable biological data from modern in vitro biology techniques, a compound need not have significant aqueous solubility. The use of screening libraries that have drug-like properties is recommended.

4.4 VEBER RULES

Another rule set was developed using a compound set of 1100 drug candidates that had rat oral bioavailability (F) measurements [5]. The delimiter for acceptable bioavailability was 20%. This compound set had measured values of oral bioavailability, the compounds were all intended to be orally administered and intestinally absorbed, and the set was purely delimited by bioavailability, not development considerations likely embodied in the rule of 5 set (e.g., pharmaceutics, chemical stability, cost of synthesis). Bioavailability is the composite result of fraction of the dose that is absorbed in the intestine plus fraction of the dose that is not cleared by first-pass metabolism in the liver and intestine. Thus, while bioavailability is a more specific assessment than Phase I to II advancement, it is still the result of multiple barriers (e.g., passive diffusion, transporter, metabolism, solubility) and performance at each of these barriers could vary from compound to compound. In addition, the oral dosing studies were sometimes done with a cyclodextrin excipient, which would tend to increase the solubility of compounds with solubility limitations.

The researchers determined which molecular properties had the most influence on oral bioavailability (F) in rats. Higher F was statistically associated with lower MW, higher lipophilicity, lower rotatable bond count (number of single bonds, not in a ring, bound to a nonterminal heavy atom, and not including amide C-N bonds), lower H-bond count (donor: any heteroatom with ≥ 1 bonded hydrogen, acceptor: a heteroatom [excluding a number of non-hydrogen bonding moieties]), and lower PSA. For example, the number of *rotatable bonds* ("nrot") was a clear indicator for the compounds with acceptable F: $\sim$65% of compounds had nrot ≤ 7, $\sim$30% of compounds had $7 <$ nrot < 10, and $\sim$20% of compounds had nrot > 10. MW was not selected as an indicator, because higher MW was also associated with higher nrot, higher PSA, and higher H-bond count, so MW was seen as more of a surrogate for other properties than as a clear cause of F. Molecular flexibility, PSA, and hydrogen bond count were selected as important determinants of oral bioavailability. Rotatable bonds can be counted manually or using software. PSA is calculated using software and is closely related to hydrogen bonding.

Compounds are more likely to have >20% rat oral bioavailability if they have:

- rotatable bonds ≤ 10
- PSA ≤ 140 Å^2, or total hydrogen bonds ≤ 12 (acceptors plus donors)