ADMET

for Medicinal Chemists

A Practical Guide

Edited by

Katya Tsaioun and Steven A. Kates

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STEVEN A. KATES

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Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

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Library of Congress Cataloging-in-Publication Data:

ADMET for medicinal chemists : a practical guide / edited by Katya Tsaioun, Steven A. Kates.
p. ; cm.
Other title: Absorption, distribution, metabolism, excretion, toxicity for medical chemists
Includes bibliographical references and index.
ISBN 978-0-470-48407-4 (cloth)
1. Drug Development. I. Tsaioun, Katya. II. Kates, Steven A., 1961- III.
Title: Absorption, distribution, metabolism, excretion, toxicity for medical chemists.
[DNLM: 1. Drug Design. 2. Drug Toxicity. 3. Pharmaceutical Preparations–chemistry.
4. Pharmacokinetics. QV 744 A238 2011]
RM301.27.A36 2011
615′.19–dc22

2010021925

Printed in Singapore

eBook: 978-0-470-91509-7
oBook: 978-0-470-91511-0
ePub: 978-0-470-92281-1

10 9 8 7 6 5 4 3 2 1

CONTENTS

Preface **xv**

Contributors **xix**

1 Introduction **1**
Corinne Kay

1.1 Introduction 1
1.2 Voyage Through The Digestive System 2
1.2.1 The Mouth 3
1.2.2 The Stomach 4
1.2.3 The Small Intestine: Duodenum 7
1.2.4 The Small and Large Intestine: Jejunum, Ileum, Colon 9
1.2.5 Hepatic-Portal Vein 13
1.3 The Liver Metabolism 15
1.3.1 CYP450 (CYPs) 17
1.4 The Kidneys 21
1.4.1 Active Tubular Secretion 23
1.4.2 Passive Tubular Reabsorption 24
1.5 Conclusions 25
References 25

2 *In Silico* ADME/Tox Predictions **29**

David Lagorce, Christelle Reynes, Anne-Claude Camproux, Maria A. Miteva, Olivier Sperandio, and Bruno O. Villoutreix

2.1 Introduction 29
2.2 Key Computer Methods for ADME/Tox Predictions 30
2.2.1 Drug Discovery 30
2.2.2 Applying or Not ADME/Tox Predictions, Divided Opinions 35
2.2.3 *In Silico* ADME/Tox Methods and Modeling Approaches 39
2.2.4 Physicochemistry, Pharmacokinetics, Drug-Like and Lead-Like Concepts 46
2.2.5 Lipophilicity 51
2.2.6 pK_a 53
2.2.7 Transport Proteins 61
2.2.8 Plasma Protein Binding 62
2.2.9 Metabolism 65
2.2.10 Elimination 67
2.2.11 Toxicity 67
2.3 Preparation of Compound Collections and Computer Programs, Challenging ADME/Tox Predictions and Statistical Methods 73
2.3.1 Preparation of Compound Collections and Computer Programs 73
2.3.2 Preparing a Compound Collection: Materials and Methods 75
2.3.3 Cleaning and Designing the Compound Collection 83
2.3.4 Searching for Similarity 85
2.3.5 Generating 3D Structures 86
2.4 ADME/Tox Predictions within Pharmaceutics Companies 86
2.4.1 Actelion Pharmaceuticals Ltd. 86
2.4.2 Bayer 86
2.4.3 Bristol-Myers Squibb 87
2.4.4 Hoffmann-La Roche Ltd. 87
2.4.5 Neurogen Corporation 87
2.4.6 Novartis 88
2.4.7 Schering AG 88
2.4.8 Vertex Pharmaceuticals 88
2.5 Challenging ADME/Tox Predictions 88
2.5.1 Tolcapone 89
2.5.2 Factor V Inhibitors 89
2.5.3 CRF-1 Receptor Antagonists 90
2.6 Statistical Methods 90
2.6.1 Principal Component Analysis 90
2.6.2 Partial Least Square 93
2.6.3 Support Vector Machine 96

2.6.4 Decision Trees 98
2.6.5 Neural Networks 101
2.7 Conclusions 104
References 105

3 Absorption and Physicochemical Properties of the NCE 125
Jon Selbo and Po-Chang Chiang

3.1. Introduction 125
3.2. Physicochemical Properties 126
3.3. Stability 127
3.4. Dissolution and Solubility 128
3.4.1. Dissolution Rate, Particle Size, and Solubility 128
3.4.2. pH and Salts 130
3.4.3. *In Vivo* Solubilization 133
3.5. Solid State 134
References 139

4 ADME 145
Martin E. Dowty, Dean M. Messing, Yurong Lai, and Leonid (Leo) Kirkovsky

4.1 Introduction 145
4.2 Absorption 146
4.2.1 Route of Administration 146
4.2.2 Factors Determining Oral Bioavailability 149
4.3 Distribution 157
4.3.1 Drug Distribution 157
4.3.2 Volume of Distribution 158
4.3.3 Free Drug Concentration 160
4.3.4 CNS Penetration 162
4.4 Elimination 165
4.4.1 Elimination Versus Clearance 165
4.4.2 Metabolism Versus Excretion 165
4.4.3 Drug-Free Fraction and Clearance 166
4.4.4 Lipophilicity and Clearance 166
4.4.5 Transporters and Clearance 166
4.4.6 Metabolism 167
4.4.7 Excretion 171
4.5 Drug Interactions 174
4.5.1 Absorption-Driven DDI 174
4.5.2 Distribution-Driven DDI 174
4.5.3 Excretion-Driven DDI 174
4.5.4 Metabolism-Driven DDI 175
4.5.5 Tools for Studying Drug Metabolism 177
4.5.6 Applications of Drug Metabolism Tools 180

4.5.7 Tools for Studying Drug Excretion 184
4.6 Strategies for Assessing ADME Properties 186
4.6.1 Assessing ADME Attributes at Different Stages of Discovery Projects 186
4.7 Tool Summary for Assessing ADME Properties 190
References 190

5 Pharmacokinetics for Medicinal Chemists 201
Leonid (Leo) Kirkovsky and Anup Zutshi

5.1 Introduction 201
5.1.1 History of Pharmacokinetics as Science 201
5.2 ADME 202
5.2.1 Absorption 202
5.2.2 Distribution 204
5.2.3 Metabolism 207
5.2.4 Excretion 207
5.3 The Mathematics of Pharmacokinetics 211
5.3.1 Compartmental Versus Noncompartmental Analysis 212
5.4 Drug Administration and PK Observations 212
5.4.1 Analysis of Intravenous PK Data 213
5.4.2 Analysis of Extravascular PK Data 227
5.4.3 Analysis of Intravenous Infusion Data 230
5.4.4 Analysis of PK Data after Multiple Dose Administrations 231
5.4.5 Analysis of PK Data after Escalating Dose Administrations 233
5.5 Human PK Projection 235
5.5.1 Allometric Scaling 235
5.5.2 Scaling by Physiologically Based Pharmacokinetic Modeling 237
5.5.3 *In Vitro–In Vivo* Correlations 239
5.6 PK Practices 239
5.6.1 PK Studies for Different Stages of Discovery Projects 240
5.6.2 Key Parameters of PK Studies 241
5.7 Engineering Molecules with Desired ADME Profile 269
5.A Appendices 269
5.A.1 General Morphinometric Data for Different Species 269
5.A.2 Organ Weights in Different Species 270
5.A.3 Organ, Tissue, and Fluid Volumes in Different Species 271
5.A.4 Blood Content in Different Rat Organs 271
5.A.5 Biofluid Flow through the Organs in Different Species 272
5.A.6 Anatomical Characteristics of GI Tract in Different Species 273
5.A.7 The pH and Motility of GI Tract in Different Species 274
5.A.8 Phase I and Phase II Metabolism in Different Species 274
Acknowledgments 277
References 277

6 Cardiac Toxicity **287**
Ralf Kettenhofen and Silke Schwengberg

6.1 Introduction 287
6.2 Ion Channel-Related Cardiac Toxicity 287
6.2.1 Cardiac Electrophysiology 288
6.2.2 Delayed Repolarization: Mechanisms and Models 290
6.2.3 Shortened Ventricular Repolarization 294
6.2.4 Alterations in Intracellular Ca^{2+} Handling 296
6.2.5 Preclinical Models for Assessment of Ion Channel-Related Cardiotoxicity 297
6.3 Nonarrhythmic Cardiac Toxicity 299
6.3.1 Definition of Drug-Induced Cardiac Toxicity 300
6.3.2 Assays for Detection of Nonarrhythmic Cardiac Toxicity 300
6.3.3 Biochemical and Molecular Basis of Drug-Induced Cardiac Toxicity—Impairment of Mitochondrial Function 304
References 306

7 Genetic Toxicity: *In Vitro* Approaches for Medicinal Chemists **315**
Richard M. Walmsley and David Elder

7.1 Introduction 315
7.1.1 Scope of this Chapter 315
7.1.2 Definitions 316
7.1.3 Positive Genotoxicity Data is not Uncommon and Very Costly 316
7.1.4 Why Genome Damage is Undesirable 317
7.1.5 The Inherent Integrity of the Genome and its Inevitable Corruption 317
7.1.6 Many Chemicals can Cause Cancer, but do not Pose a Significant Risk to Humans 318
7.1.7 The False Positives: Many Chemicals Produce Positive Genotoxicity Data that are neither Carcinogens nor *In Vivo* Genotoxins 318
7.1.8 Defense Against Genotoxic Damage 319
7.1.9 Mechanisms of Genotoxic Damage 320
7.1.10 Genotoxicity Assessment Occurs after Medicinal Chemistry Optimization 321
7.2 Limitations in the Regulatory *In Vitro* Genotoxicity Tests 322
7.2.1 Biology Limitations of *In Vitro* Tests 322
7.2.2 Hazard and Safety Assessment have Different Requirements 323
7.2.3 The Data from Genetic Toxicologists 323
7.3 Practical Issues for Genotoxicity Profiling 324
7.3.1 Vehicle 324

7.3.2 Dilution Range 324
7.3.3 Purity 324
7.4 Computational Approaches to Genotoxicity Assessment: The *In Silico* Methods 325
7.4.1 General Considerations 325
7.4.2 The Chemistry of Genotoxins 328
7.5 Genotoxicity Assays for Screening 335
7.5.1 Bacterial Gene Mutation Assays 337
7.5.2 Mammalian Cell Mutation Assays 338
7.5.4 Chromosome Damage and Aberration Assays 339
7.5.5 The "Comet" Assay 340
7.5.6 DNA Adduct Assessment 341
7.5.7 Gene Expression Assays 341
7.6 The "Omics" 343
7.7 Using Data from *In Vitro* Profiling: Confirmatory Tests, Follow-Up Tests, and the Link to Safety Assessment and *In Vivo* Models 343
7.7.1 Annotations from Screening Data 344
7.7.2 Can a Genetic Toxicity Profile Assist with *In Vivo* Testing Strategies? 344
7.8 What to Test, When, and How 345
7.9 Changes to Regulatory Guidelines Can Influence Screening Strategy 346
7.10 Summary 347
Acknowledgment 347
References 348

8 Hepatic Toxicity 353
Jinghai James Xu and Keith Hoffmaster

8.1 Introduction 353
8.2 Mechanisms of DILI 354
8.2.1 Reactive Metabolite Formation 355
8.2.2 Mitochondrial Dysfunction and Oxidative Stress 357
8.2.3 Bile Flow, Drug-Induced Cholestasis, and Inhibition of Biliary Efflux Transporters 359
8.3 Assays and Test Systems to Measure Various Types of DILI 360
8.4 Medicinal Chemistry Strategies to Minimize DILI 365
8.5 Future Outlooks 370
Acknowledgment 370
References 370

9 *In Vivo* Toxicological Considerations 379
John P. Devine, Jr.

9.1 Introduction 379
9.2 Route of Administration 379

9.2.1 Oral Route 380
9.2.2 Intravenous Route 381
9.2.3 Dermal Route 382
9.3 Formulation Issues 383
9.4 Compound Requirements 384
9.5 Animal Models 385
9.5.1 Mouse 385
9.5.2 Rat 386
9.5.3 Dog 386
9.5.4 Swine 386
9.5.5 Nonhuman Primates 387
9.6 IND-Supporting Toxicology Studies 387
9.6.1 Single-Dose Studies 387
9.6.2 Repeat-Dose Studies 388
9.7 Study Result Interpretation 392
9.7.1 Clinical Observations 392
9.7.2 Body Weight/Feed Consumption 393
9.7.3 Clinical Pathology 393
9.7.4 Clinical Chemistry 393
9.7.5 Electrocardiograms 394
9.7.6 Organ Weights 394
9.7.7 Pathology 395
9.8 Genetic Toxicology Studies 395
9.8.1 Gene Mutation 395
9.8.2 Chromosomal Aberration 396
9.8.3 *In Vivo* Mouse Micronucleus 396
9.9 Conclusion 396
References 397

10 Preclinical Candidate Nomination and Development 399
Nils Bergenhem

10.1 Introduction 399
10.2 Investigational New Drug Application and Clinical Development 400
10.2.1 Chemistry, Manufacturing, and Control Information 401
10.2.2 Animal Pharmacology and Toxicology Studies 401
10.2.3 Clinical Protocols and Investigator Information 401
10.3 Strategic Goals for the Preclinical Development 402
10.4 Selection of Preclinical Development Candidate 403
10.4.1 Efficacy 403
10.4.2 Safety/Tolerance 405
10.4.3 PK 407
10.4.4 Non-GLP Toxicological Study 407

10.5 CMC 408
10.5.1 Solubility 408
10.5.2 Solutions Stability 408
10.5.3 Synthetic Feasibility, Solid-State Stability, and Hygroscopicity 408
10.5.4 Patent Position 408
10.6 Preclinical Studies 409
10.6.1 Example 1: IND Enabling Data Package to Support 1 Month Dosing in Man 410
10.6.2 Example 2: Peroxisome Proliferator-Activated Receptor Agonist for Type-2 Diabetes 410
10.6.3 Mass Balance 410
10.6.4 Animal Pharmacology and Toxicology Studies 410
10.6.5 Regulatory 414
10.7 Conclusions 415
References 415

11 Fragment-Based Drug Design: Considerations for Good ADME Properties 417
Haitao Ji

11.1 Introduction 417
11.2 Fragment-Based Screening 418
11.2.1 Fragment Library Design 419
11.2.2 Detection and Characterization of Weakly Binding Ligands 420
11.2.3 Approaches from Fragment to Lead Structures 427
11.3 Case Studies of Fragment-Based Screening for Better Bioavailability 431
11.3.1 Adenosine Kinase 431
11.3.2 Leukocyte Function-Associated Antigen-1 432
11.3.3 Matrix Metalloproteinase 3 (Stromelysins) 432
11.3.4 Protein Tyrosine Phosphatase 1B 433
11.3.5 β-Secretase (BACE-1) 436
11.3.6 SH2 Domain of pp60Src [62, 129] 439
11.3.7 Thrombin 439
11.3.8 Urokinase 441
11.3.9 Cathepsin S 442
11.3.10 Caspase-3 442
11.3.11 HIV-1 Protease 444
11.4 *De Novo* Design 445
11.4.1 *In Silico* Fragment Screening 447
11.4.2 Scaffold Hopping 448

11.5 Case Studies of *De Novo* Design for Better Bioavailability 450
11.5.1 DNA Gyrase 450
11.5.2 Factor Xa 450
11.5.3 X-Linked Inhibitor of Apoptosis Protein 451
11.5.4 Activator Protein-1 [196b] 451
11.6 Minimal Pharmacophoric Elements and Fragment Hopping 452
11.6.1 Minimal Pharmacophoric Elements 452
11.6.2 Fragment Hopping 453
11.6.3 Case Study: Nitric Oxide Synthase 457
11.7 Conclusions and Future Perspectives 459
Acknowledgments 460
References 460

Index **487**

PREFACE

Medicinal chemistry and drug development have undergone a rapid evolution since the 1990s due to a much expanded biological and chemical toolbox allowing novel target identification and rapid synthesis of a large number of diverse chemical libraries. Despite this progress, substantial increases in R&D expenditures, and an ever-increasing number of molecules screened and synthesized every year, the pharmaceutical industry has found itself in a productivity paradox. While these improvements should have significantly increased the number of new molecular entities (NMEs) identified each year, the actual numbers in this period remained at a low and constant level with high attrition rates at both the early and the late stages of clinical development.

We believe a number of factors have contributed to this paradox.

One factor is the current system of academic training whose objectives of instructing scientists are not always aligned with the objectives of the pharmaceutical industry. The discovery of novel therapeutics is an inherently complex and interdisciplinary process, requiring close integration of scientists from several disciplines in an environment in which lessons are shared and taught across an organization. Current models of academic training emphasize specialization and insufficiently address the need to understand related subjects.

Not surprisingly, drug-discovery organizations frequently experience difficulties integrating the efforts of chemists, biologists, and preclinical and clinical development specialists. This leaves each discipline producing scientifically valid work that may often have little relevance for developing a commercial product. This book is a product of a collaborative work of a medicinal chemist and an ADMET scientist; however, the bulk of the content of this book was written by experts in specialized subsegments of these fields. The structure of this book mirrors the most successful

organizational model for drug discovery: collaboration and communication among team members comprising a variety of specialties.

While medicinal chemists collaborate among themselves, successful programs depend upon these drug developers to interact with specialists from other disciplines to commercialize therapeutic agents. This is the key insight *ADMET for Medicinal Chemists* provides; the techniques are just a "*how-to*."

The acronym "ADMET" refers to "absorption, distribution, metabolism, excretion, and toxicity." These parameters, in addition to efficacy, are critical in determining whether an NME will become a clinical candidate and subsequently a commercially viable product. Depending on context, the acronym can refer either to the properties of a compound, the process of determining those properties, or the discipline that focuses on that process.

"Early ADMET" is a discipline that emerged in the late 1990s. The field has created a unique interdisciplinary interface between medicinal chemists, biologists, formulators, toxicologists, and preclinical development scientists. Consequently, for medicinal chemists, early ADMET is the ideal entry point for expanding the understanding to related disciplines.

The advent of early ADME profiling of drug candidates in a high-throughput fashion in conjunction with proof-of-principle biological efficacy optimization has reduced drug failures in clinical trials due to ADME/DMPK reasons from 40 to $<7\%$ during the 1980s–1990s to late 2000s. Even though drug-discovery productivity failed to improve during this period, the implementation of early ADMET was indisputably a major success. The goal of an ADMET program is to guide candidate selection through the early identification of molecules with suboptimal properties so that their corresponding technical issues could be addressed before large development costs have been incurred. The goal of this book is to guide medicinal chemists in how to implement early ADMET testing in their workflow in order to improve both the speed and efficiency of their efforts.

Many medicinal chemists are unfamiliar with the pharmacological pathway of a drug administered orally. Their chemical innovation can be improved by a better understanding of the digestive system which is provided in Chapter 1. Topics such as enterohepatic circulation, permeability, P-glycoproteins, microsomal stability, first-pass, and glomerular filtration are described.

Structure-based *in silico* ADMET screening models and software approaches are often used to guide medicinal chemistry efforts to design molecules with desired properties. Lipinski, Veber, and Oprea have developed rules that describe the relationship between a compound and its corresponding ADMET properties. These rules have been built into software. Chapter 2 outlines the key computer methods, such as rule-based methods, QSAR, and machine learning approaches. The chapter also discusses and compares the commercially available programs and demonstrates methods for implementation to prepare compound collections.

The physicochemical properties of a new chemical entity (NCE) can impact absorption and pharmacokinetics. Chapter 3 summarizes the strategies for applying *in silico* filters for optimization, and the impact of the solid state of the molecule on physicochemical properties of NCEs in medicinal chemistry.

Chapter 4 provides an overview of liver metabolic stability, plasma stability, solution stability, plasma protein binding, intestinal, blood–brain barrier, tissue distribution, permeability models, excretion (biliary and renal), CYP450 inhibition, and efflux and uptake transporters. These parameters are important because physiological phenomena and the properties of drug candidates in biological systems are based upon the disposition and metabolism of a NCE. The chapter also discusses the various preclinical tools that may be used to predict human performance, and strategies for prioritizing and conducting these assays in a lead optimization program.

Pharmacokinetics (PK) describes what the body does to the drug and is dependent on the dose administered, site of administration, and the physiological state of the organism. PK expresses the rates of disposition (movement) of a drug when administered to a living organism such as (A)bsorption, (D)istribution, (M)etabolism, and (E)xcretion (ADME). Chapter 5 provides a brief overview of basic pharmacokinetic principles such as T_{max}, C_{max}, V_d (volume of distribution), AUC (area under the curve), $t_{1/2}$ (half-life), bioavailability, extraction ratio, and metabolic clearance. The inevitable formulas that explicate the concepts are conceptually and pictorially described and explained.

Toxicity assays are the most critical tests of an NCE performed prior to its administration into humans. These tests are designed to ensure the safety of the first human subjects. The FDA requires many different assays for achieving this essential objective. These assays examine how a compound affects different aspects of human pharmacology. Chapter 6 discusses the fundamentals and mechanisms of cardiac safety including hERG, other ion channels, and nonion channel related cardiac toxicity. Chapter 7 outlines genetic toxicity, including AMES, micronucleus, and GreenScreen. Chapter 8 describes hepatic toxicity, including necrosis, steatosis, cholestasis, reactive metabolites, and covalent binding.

The FDA requires for an NCE *in vivo* toxicological assessment of typically two species: one rodent and one nonrodent. Chapter 9 considers the route of administration for the intended therapeutic (bolus versus infusion and potential inadvertent routes), and the compound requirements for toxicological studies as related to different species. Chapter 9 also covers formulation issues, such as overage, spillage, stability, reactivity/compatibility with glassware, infusion equipment, method validation, and sample analysis. IND-enabling studies and species selection for different therapeutic indications are also reviewed.

Preclinical candidate nomination and development are discussed in Chapter 10, which provides an overview of the late stages of the discovery process, including the process of selecting preclinical candidates, and designing appropriate preclinical studies to support an IND.

Novel approaches to drug design constantly evolve. Chapter 11 addresses fragment-based drug design, such as minimal pharmacophoric elements and fragment hopping, which are recent innovative tools developed for designing drug candidates.

We wish to thank the contributors to this book. They have succeeded in describing the importance for a medicinal chemist to understand ADMET. We are appreciative of their support and substantial effort in providing their expertise and thoughtfulness to this project.

We hope that this book provides readers with an appreciation for the complexity of designing and developing new therapeutic agents for human clinical trials and their subsequent approval into the marketplace. We are privileged to be able to work in a field with such boundless opportunities for reducing human suffering and it is our obligation to create the best of these opportunities.

Color representations of selected figures in this book can be found at ftp://ftp.wiley.com/public/sci_tech_med/admet

KATYA TSAIOUN
STEVEN A. KATES

Watertown, Massachusetts
Maynard, Massachusetts
October 2010

CONTRIBUTORS

Nils Bergenhem, Vice President & CSO, CPEX Pharmaceuticals, Inc., 2 Holland Way, Exeter, NH, USA

Anne-Claude Camproux, UMRS 973 Inserm-Paris Diderot, Paris, France

Po-Chang Chiang, Pfizer Inc., Chesterfield Parkway West, Chesterfield, Missouri

John P. Devine, Jr., Basi Mount Vernon, Indiana

Martin E. Dowty, Pfizer Inc., Chesterfield Parkway West, Chesterfield, Missouri

David Elder, GalaxoSmithKline Research and Development, Externalisation Group, PCD, Ware, United Kingdom

Keith Hoffmaster, Novartis Institutes for Biomedical Research, Cambridge, Massachusetts

Haitao Ji, Department of Chemistry, University of Utah, Salt Lake City, Utah, USA

Corinne Kay, Med-Simple, Hillside Farm, Keir, Dunblane, Scotland

Ralf Kettenhofen, AXIOGENESIS AG, Nattermannallee, Cologne, Germany

Leonid (Leo) Kirkovsky, Pfizer Inc., Chesterfield Parkway West, Chesterfield, Missouri

David Lagorce, UMRS 973 Inserm-Paris Diderot, Paris, France

Yurong Lai, Pfizer Inc., Chesterfield Parkway West, Chesterfield, Missouri

Dean M. Messing, Pfizer Inc., Chesterfield Parkway West, Chesterfield, Missouri

Maria A. Miteva, UMRS 973 Inserm-Paris Diderot, Paris, France

Christelle Reynes, UMRS 973 Inserm-Paris Diderot, Paris, France

Silke Schwengberg, AXIOGENESIS AG, Nattermannallee, Cologne, Germany

Jon Selbo, SSCI, A Division of Aptuit, West Lafayette, Indiana

Olivier Sperandio, UMRS 973 Inserm-Paris Diderot, Paris, France

Bruno O. Villoutreix, UMRS 973 Inserm-Paris Diderot, Paris, France

Richard M. Walmsley, Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom

Jinghai James Xu, Merck Research Labs, West Point, Pennsylvania

Anup Zutshi, Pfizer Inc., Chesterfield Parkway West, Chesterfield, Missouri

1

INTRODUCTION

Corinne Kay

1.1 INTRODUCTION

Absorption, distribution, metabolism, and excretion (ADME) properties have been and still are a significant reason for attrition in drug discovery. Paradoxically, medicinal chemists have to solve complex ADME issues with a solid organic chemistry background and scant anatomy or physiology training. In reality, drug metabolism is not any different from daily food digestion and understanding of human nutrition demystifies many drug metabolism reactions. Grasping the logical sequence of each step in the digestion process as well as the nature of the chemical reactions that occur in each organ contributes to the understanding of food digestion and drug metabolism.

The human digestive system processes on average 30 tons of food in a lifetime. Food digestion provides nutrients for the body's function and repair. Useful nutrients are stored in a form that is compatible with existing biological systems (e.g., fat, glycogen). It produces and recycles complex molecules such as bile acids and catabolizes fats. More importantly, the liver is set up to recognize and destroy toxic entities and does so with relentless efficiency.

In order to carry out such a vast array of chemical reactions in a highly compact space, the human body effectively runs the process in set stages corresponding to the various organs of the digestive system. Each organ possesses its own controlled pH. An associated battery of enzymes as well as coenzymes are activated at this pH but denatured at another pH at the next stage. Ingested food will spend from minutes (mouth) to hours (gut) in a single organ where it is effectively mechanically stirred at 37°C in an enzyme bath at a set pH. After all possible chemical transformations

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have occurred in that organ, it then moves to the next organ in the digestive tract. Fascinatingly, the associated pH change causes the incoming enzymes to denature, thus exposing the partially processed food to a new set of reaction conditions and enabling new chemical transformations to take place. Little wonder that after meandering for 12–48 h through the 8–9 m length of the digestive track that most complex chemicals have been transformed (or metabolized) to their constituent building blocks. Since the purpose of eating is not to dispose of food waste but to bring nutrients to our body, these chemical building blocks (amino acids, sugars, etc.) are carried to and then stored in appropriate cells in the human body.

Not surprisingly, a drug entering the digestive system will be exposed to the same environment as food and will be subjected to the same battery of chemical reactions. For a patient ingesting a tablet, the constituent chemicals will be exposed to the same mechanical stirring at 37°C, the same pH as well as enzymes. The chemical transformations that occur during food digestion are free to act on the constituents of the drug. Since most drugs are presented to the body as solid or semisolid dosage forms, the drug particles must first be released from that form and dissolved. Furthermore, drug absorption, whether from the gastrointestinal (GI) tract or other sites, requires the passage of the drug in a molecular form across the barrier membrane. The drug will be required to possess the desirable biopharmaceutical properties enabling it to pass from a region of high concentration to a region of low concentration across the membrane into the blood, or general circulation.

Insight to this process is a key to understanding many drug metabolism concepts. This introduction chapter is not intended to be a physiology textbook. It demonstrates the link between nutrition and drug metabolism and thus provides a framework upon which medicinal chemists can apply their extensive organic chemistry knowledge to solve the ever occurring ADME issues. It has been said that "anyone can contribute solutions provided the problem is explained in a language they understand." Aspiring and even experienced medicinal chemists are encouraged to take basic nutrition and physiology courses to deepen their understanding of developed drugs exposure to these environments.

1.2 VOYAGE THROUGH THE DIGESTIVE SYSTEM

It takes 12–48 h for ingested food to complete its voyage through the digestive system. The food first arrives in the mouth (pH = 7) where it is chewed and exposed to its first set of enzymes, called salivary enzymes. Subsequently, the food travels down the esophagus to the stomach (pH = 1–2), which undergoes receptive relaxation as it fills to a capacity of 2 L or more (Figure 1.1). The next stage in the digestive system is the small intestine where chemical digestion continues and most nutrients are absorbed. The small intestine is made up of three parts: the duodenum where the contents of the stomach are neutralized to pH = 6 by pancreatic secretions ($NaHCO_3$), then the 2 m long jejunum (pH = 7–8), and finally the ileum (3 m long). Those substances which are not digestible or not absorbed join the large intestine, where leftovers are formed into semisolid masses ready for disposal. When digested nutrients are absorbed

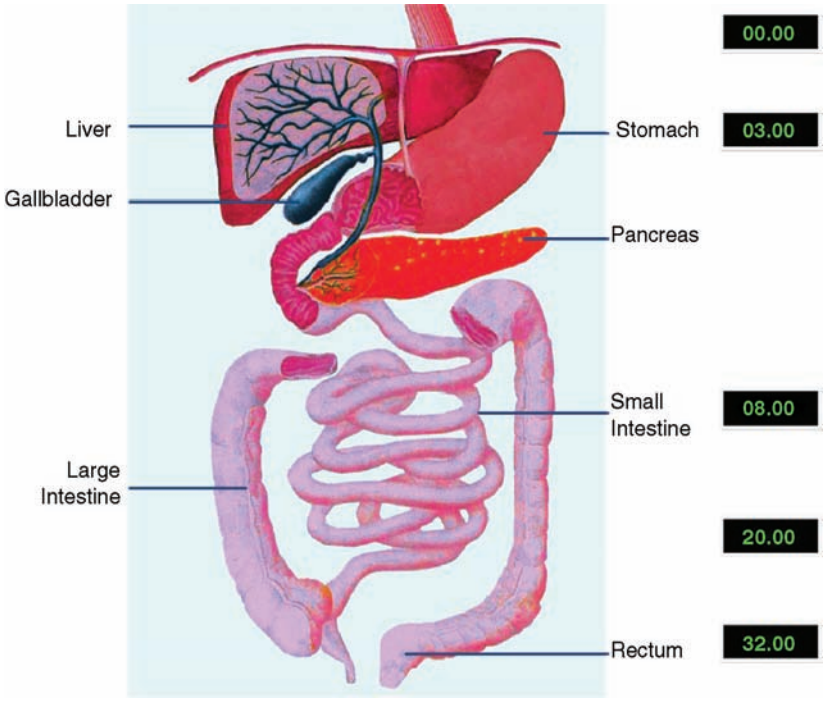

Figure 1.1 The digestive system. The numbers to the right of the diagram represent the elapsed time.

(i.e., exit the gut into the blood stream), they are ducted to the liver via the hepatic-portal vein (HPV) (pH = 7.4). Following metabolism in the liver, molecules can either be ducted back into the digestive system via the bile (enterohepatic cycling) or finally enter the general blood circulation. Finally, approximately 25% of total systemic blood flow is diverted through the kidneys, which act as an on-line filtration unit, and water-soluble waste is concentrated in the urine.

The various events occurring during drug metabolism are more easily understood by visualizing the sequence "mouth—esophagus—stomach—duodenum—gut—liver—kidneys" as well as understanding the chemical reactions taking place in each organ.

1.2.1 The Mouth

The mouth is the reception center of the digestive system and the place where food is initially processed before being passed onto the stomach via the esophagus. In the mouth, mechanical as well as chemical digestion occurs. On average 1 L of saliva is secreted at various points into the mouth through salivary glands. Saliva contains salivary enzymes, which include amylases, which break down complex carbohydrates into simple sugars such as maltose and glucose, and peroxidases. Some

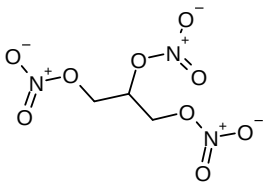

Figure 1.2 Structure of GTN (glycerin trinitrate).

lipases are also present in the saliva, resulting in some of the lipid (i.e., fat or dietary triglycerides) digestion starting to occur in the mouth. Additionally, aspartate aminotransferase (AST), alanineamino transferase (ALT), lactate dehydrogenase (LDH), and acidic and alkaline phosphatase have been reported to be released from the normal and especially damaged cells of periodontal tissues into saliva. Saliva also contains immunoglobin A (IgA), an antibody playing a minor role in human immunity.

Some nutrients are capable of crossing the mucosa and the membranes that line the cavity of the mouth and are captured by the profusion of capillaries to enter the bloodstream. This avenue is used by hypoglycemic patients to quickly deliver glucose to raise their glucose blood levels by placing a glucose tablet under their tongue. The same principle is used when small molecular weight, water-soluble drugs such as glycerin trinitrate, or nitroglycerin (GTN) (Figure 1.2) are given *via* the sublingual route to patients suffering from angina. This ensures the rapid entry of the drug into the bloodstream as well as bypasses the remainder of the digestive system including the liver where it would otherwise be extensively metabolized.

The same route of administration has been exploited by Generex Biotechnology Corporation [1] to develop Oral-lyn™. This formulation delivers insulin as a fine spray in the buccal (i.e., mouth) cavity. The peptide drug is rapidly absorbed through the mucosal lining of the mouth and enters the bloodstream, where it is reported to produce glucodynamic profiles comparable to that produced by injection of regular human insulin. This drug was first approved for use in Ecuador in 2005 [2] and recently received approval in a number of other countries. The buccal administration of insulin also assures that the drug does not enter the lungs and, therefore, is free of pulmonary side-effect associated with inhaled insulin products.

Similarly, the anticoagulant drug heparin, a polysaccharide not suitable for oral administration, is given to patients *via* the subcutaneous route. Its similarity to starch makes it an obvious target to amylases (Figure 1.3).

1.2.2 The Stomach

The food bolus is swallowed and enters the stomach where both chemical digestion as well as absorption (from the stomach into the bloodstream) occurs. The wall of the stomach is lined with millions of gastric glands, which together secrete 400–800 mL of gastric juice at each meal. Several types of cells are found in the gastric glands

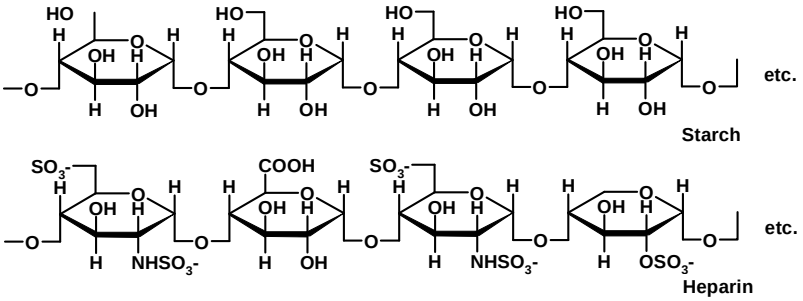

Figure 1.3 The similarities between the structures of starch and heparin.

including parietal cells, chief cells, mucus-secreting cells, and hormone-secreting (endocrine) cells.

1.2.2.1 Chemical Digestion Claude Bernard (1813–1878), known as the founder of experimental physiology, demonstrated that contrary to popular belief, little digestion occurs in the stomach. This organ only processes up to half the carbohydrates in a meal, one-tenth of the protein, and hardly any fat while the bulk of the digestion takes place in the intestines. The stomach effectively acts as a food mixer and acid-and-enzyme bath and the breadth of chemical reactions that ensue are of importance to the medicinal chemist. Every few minutes its strong, muscular walls undergo a spasm of squeezing to churn the food into semiliquid state called chyme. Parietal cells secrete hydrochloric acid (HCl) and intrinsic factor. Intrinsic factor is a protein that binds ingested vitamin B12 and enables it to be absorbed by the intestine. A deficiency of intrinsic factor, as a result of an autoimmune attack against parietal cells, causes pernicious anemia. Chief cells synthesize and secrete pepsinogen, the precursor to the proteolytic enzyme pepsin. Pepsin cleaves peptide bonds, favoring those on the C-terminal side of tyrosine, phenylalanine, and tryptophan residues. Its action breaks long polypeptide chains into shorter lengths. Secretion by the gastric glands is stimulated by the hormone gastrin, which is released by endocrine cells in the stomach in response to the arrival of food. Gastrin stimulates the production of hydrochloric acid (HCl), reducing the pH to 1–2, which inactivates amylases, swallowed with the saliva, and denatures ingested proteins, making them more vulnerable to attack by pepsins. Although most of the lipases are secreted from the pancreas into the duodenum, some lipases are present in the stomach and perform an ester bond hydrolysis on a limited range of lipids to produce fatty acids and glycerol.

This panoply of chemical reactions highlights the breadth and variety of the chemical transformations that can occur in the stomach and provide a warning to medicinal chemists attempting to design compounds of peptidic nature or containing multiple amide bonds and unhindered alkyl esters.

1.2.2.2 Absorption Only a limited range of substances are actually absorbed through the stomach lining into the blood. The stomach can absorb glucose and other

simple sugars, amino acids, and some fat-soluble substances. A number of alcohols, including ethanol, are readily absorbed from the stomach. Water moves freely from the gastric contents across the gastric mucosa into the blood. In tracer experiments [3] using deuterium oxide, about 60% of the isotopic water placed in the stomach was absorbed into the blood in 30 min. The net absorption of water from the stomach is small because water readily moves from the blood across the gastric mucosa to the lumen of the stomach. The absorption of both water and alcohol can be slowed down if the stomach contains food, especially fat, presumably since gastric emptying is delayed and most water is absorbed from the jejunum.

From a medicinal chemistry point of view, the persistent issue associated with the stomach is that of solubility. Only compounds in solution are available for permeation across the gastric membrane and solubility of drug molecules at pH = 2 is often an issue. The following examples illustrate the significance of this problem.

Salicylic acid (pK_a 3.5 and 13.4) is weakly acidic and only 30% is ionized at pH = 2. Its insolubility precludes its absorption in the stomach (Figure 1.4). It is soluble in the intestine (pH = 6.4), but since it is present in its ionized form, it is unable to be effectively absorbed through the gut wall. Given that salicylic acid is also a stomach irritant, it is prepared as an ester prodrug to reduce the amount of acid actually in contact with the gut lining. When it reaches the blood where it is 50–90% plasma bound (depending on the concentration), it is processed by esterases and is converted back to its active form (salicylic acid).

Acid-reducing agents including omeprazole are widely used [4] by patients with HIV to treat acid reflux disease, heartburn, and stomach ulcers. In a recent study [5], 18 HIV-negative volunteers were given ritonavir-boosted saquinavir along with omeprazole for 15 days. Results showed that the addition of omeprazole caused an 82% increase in the levels of saquinavir (invirase) in the blood. It was argued that omeprazole is unlikely to increase saquinavir levels by its weak inhibition of the major liver enzyme CYP3A4 that breaks down saquinavir. However, the most plausible explanation was that saquinavir is dissolving more readily in a less-acidic environment.

The pH of the gastric contents controls the absorption of certain ionizable materials such as aspirin, which is readily absorbed in its unionized form when the stomach is acidic, but more slowly when gastric contents are neutral.

A number of solubility tests are available to assist in identifying this issue prior to drug administration to humans, and these are discussed in subsequent chapters. In addition to the usual solubility tests, many groups [6] have reported the use of the fasted and fed-state simulated gastric fluid (SGF) test [7] due to its more relevance to a physiological environment.

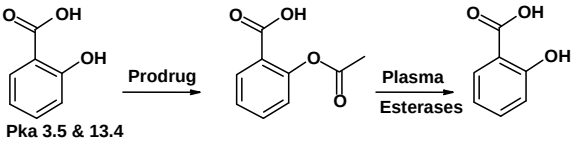

Figure 1.4 Aspirin—solubility and ionization.

1.2.3 The Small Intestine: Duodenum

Following stomach peristalsis and digestion, the pyloric sphincter relaxes and allows the food (and ingested drugs) to enter the first part of the small intestine, the duodenum. The duodenum [8] is a 20 cm long smooth muscle lined tube (Figure 1.5). Two ducts enter the duodenum: one of them drains the gallbladder and hence the liver, and the other drains the exocrine portion of the pancreas. Both organs produce secretions that enable further chemical digestion and have an impact on drug design. The pancreas consists of clusters of endocrine cells (the islets of Langerhans) and exocrine cells whose secretions drain into the duodenum.

1.2.3.1 Pancreatic Juices Since the pHs of the stomach and the intestine are very acidic and nearly neutral, respectively, the pancreas produces 1.5 L/day of alkaline juices (e.g., bicarbonate) to neutralize the partially acidic digested chyme. The neutralization is carried out at a slow, controlled rate and has the additional effect of denaturing incoming stomach enzymes and rendering them inactive. The secretion of pancreatic fluid is controlled by two hormones—secretin and cholecystokinin (CCK). Secretin mainly affects the release of sodium bicarbonate and CCK stimulates the release of the digestive enzymes.

Pancreatic fluid also contains a number of digestive enzymes. Most carbohydrate digestion occurs in the duodenum and is performed by pancreatic amylase, which hydrolyzes starch into a mixture of maltose and glucose. Pancreatic lipase hydrolyzes ingested fats into a mixture of fatty acids and monoglycerides. Its action is enhanced by the detergent effect of bile. In April 1999, the FDA approved orlistat as a treatment for obesity. Orlistat inactivates pancreatic lipase. About one-third of ingested fats fail to be broken down into absorbable fatty acids and monoglycerides and simply passes out in the feces.

The four "zymogens" (proteins that are precursors to active proteases) secreted from the pancreas are trypsin, chymotrypsin, elastase, and carboxypeptidase. These are immediately converted into the active proteolytic enzymes. Trypsin cleaves peptide bonds on the C-terminal side of arginine and lysine. Chymotrypsin cuts amide bonds on the C-terminal side of tyrosine, phenylalanine, and tryptophan

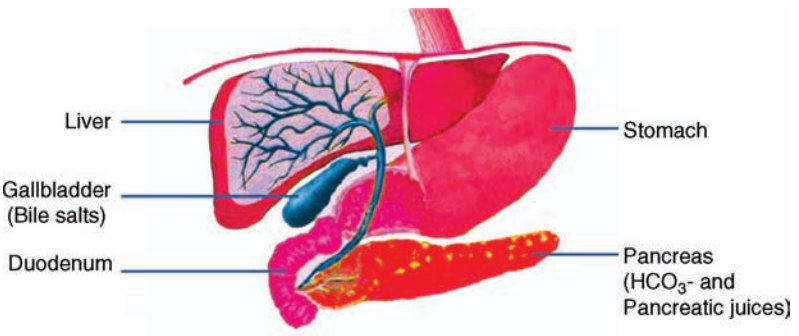

Figure 1.5 The duodenum receives input from the pancreas and the liver.

residues (the same bonds as pepsin, whose action ceases when $NaHCO_3$ raises the pH of the intestinal contents). Elastase cuts peptide bonds next to small, uncharged side-chains such as those of alanine and serine. Trypsin, chymotrypsin, and elastase are members of the family of serine proteases. Chymotrypsin precipitates hydrophilic kappa casein in milk by breaking the bond between phenylalanine (105) and methionine (106) to produce two insoluble fragments resulting in the milk curdling, thus slowing down its digestion. Finally, carboxypeptidase removes, one by one, the amino acids at the C-terminal of peptides. Carboxypeptidase A cleaves carboxyl terminal amino acids that have aromatic or aliphatic side-chains, and carboxypeptidase B cleaves carboxyl terminal amino acids that have basic side-chains. It is the presence of this wide array of enzyme proteases, which precludes the oral administration of protein or peptide drugs such as corticotrophin, vasopressin, and insulin. These would be rapidly degraded in the digestive tract and are not generally given orally. Some microencapsulation and nanoparticle formulation studies have been carried out in an attempt to circumvent these issues and are showing promise.

Diarrhea, a side-effect commonly associated with highly active antiretroviral therapy (HAART), has been ascribed to the inhibition of pancreatic lipases by protease inhibitors such as agenerase, norvir, and fortovase. An *in vitro* study [9] showed that the protease inhibitor agenerase formulated as a solution or a capsule exhibited complete inhibition of pancreatic lipase at physiological concentration. Norvir and fortovase produced 72% and 75% inhibition, respectively, at physiological concentration, as calculated from the plots to determine IC_{50} values.

Erythromycin stearate USP (ethryl) is the stearic acid salt of erythromycin. It is a crystalline powder that is practically insoluble in water. Similar to erythromycin base, the stearate is acid labile. It is thus film-coated [10] to protect it from acid degradation in the stomach and in the alkaline pH of the duodenum, where the free base is liberated from the stearate and absorbed.

1.2.3.2 Hepatic Bile The human liver produces 400–800 mL of hepatic bile each day. The bile (pH = 7.8–8.6) is then concentrated fivefold and stored in the gallbladder between meals. When food, especially containing fat, enters the duodenum, the release of the hormone CCK stimulates the gallbladder to contract and discharge its bile into the duodenum. The main constituents of bile are bile salts, bilirubin, bile pigments (end products of hemoglobin breakdown), and electrolytes. Bile salts are amphiphilic steroids, which emulsify ingested fat. The hydrophobic portion of the steroid dissolves in the fat while the negatively charged side-chain interacts with water molecules. The mutual repulsion of these negatively charged droplets keeps them from coalescing. Thus, large globules of fat (liquid at body temperature) are emulsified into tiny droplets (about 1 μm in diameter) that can be more readily digested and absorbed.

The molecules responsible for fat dispersion are bile salts such as glycocholic acid (Figure 1.6). Bile acids are facial amphipathic since they contain both hydrophobic (lipid soluble) and polar (hydrophilic) faces. The cholesterol-derived portion of a bile acid has one face that is hydrophobic (methyl groups) and one that is hydrophilic (hydroxyl groups); the amino acid conjugate is polar and hydrophilic.