

CONTENTS

Preface	xi
Contributors	xiii
1 <i>In Vitro</i> Evaluation of Metabolic Drug–Drug Interactions: Concepts and Practice	1
<i>Albert P. Li</i>	
1.1 Introduction, 2	
1.2 Mechanisms of Adverse Drug–Drug Interactions, 4	
1.2.1 Pharmacological Interactions, 4	
1.2.2 Pharmacokinetic Interactions, 5	
1.3 Drug Metabolism, 5	
1.3.1 Phase I Oxidation, 5	
1.3.2 Phase II Conjugation, 5	
1.4 CYP Isoforms, 7	
1.5 Human <i>In Vitro</i> Experimental Systems for Drug Metabolism, 7	
1.5.1 Hepatocytes, 8	
1.5.2 Liver Postmitochondrial Supernatant (PMS), 9	
1.5.3 Human Liver Microsomes, 9	
1.5.4 Recombinant P450 Isoforms (rCYP), 9	
1.5.5 Cytosol, 9	
1.6 Mechanisms of Metabolic Drug–Drug Interactions, 9	

- 1.7 Mechanism-Based Approach for Evaluation of Drug–Drug Interaction Potential, 10
 - 1.7.1 Metabolic Phenotyping, 11
 - 1.7.2 Evaluation of Inhibitory Potential for Drug-Metabolizing Enzymes, 11
 - 1.7.3 Induction Potential for Drug-Metabolizing Enzymes, 11
- 1.8 Experimental Approaches for *In Vitro* Evaluation of Drug–Drug Interaction Potential, 11
 - 1.8.1 Study 1: Metabolic Phenotyping 1—Metabolite Identification, 11
 - 1.8.2 Study 2: Metabolic Phenotyping 2—Identification of Major Metabolic Pathways, 12
 - 1.8.3 Study 3: Metabolic Phenotyping 3—Identification of P450 Isoform Pathways (P450 Phenotyping), 13
 - 1.8.4 Study 4: CYP Inhibitory Potential, 16
 - 1.8.5 Study 5: Enzyme Induction Potential, 19
 - 1.8.6 Study 6: *In Vitro* Empirical Drug–Drug Interactions, 22
- 1.9 Data Interpretation, 22
 - 1.9.1 Pathway Evaluation, 22
 - 1.9.2 P450 Inhibition, 23
 - 1.9.3 P450 Induction, 24
- 1.10 Conclusion, 25
- References, 26

2 *In Vitro* Approaches to Anticipating Clinical Drug Interactions

31

Laurie P. Volak, David J. Greenblatt, and Lisa L. von Moltke

- 2.1 *In Vitro* Systems for Human CYP450 Metabolism, 32
 - 2.1.1 Incubation Buffer (pH and Ionic Strength), 33
 - 2.1.2 MgCl₂ and Cytochrome *b*₅, 34
 - 2.1.3 Nonspecific Binding, 34
 - 2.1.4 Organic Solvents and Excipients, 35
- 2.2 Analysis of Data from *In Vitro* Systems, 36
 - 2.2.1 Linear Transformation of Michaelis–Menten Equation (Lineweaver–Burk and Eadie–Hofstee), 36
 - 2.2.2 Nonlinear Regression Analysis of Hyperbolic Kinetic Data, 37
 - 2.2.3 Consideration of Non-Michaelis–Menten Kinetics, 37
- 2.3 Use of *In Vitro* Kinetic Data to Predict *In Vivo* Clearance, 39
 - 2.3.1 Calculation of *In Vitro* (Predicted) Hepatic Clearance, 40
 - 2.3.2 Comparison of *In Vitro* (Predicted) with *In Vivo* Hepatic Clearance, 41
- 2.4 Use of *In Vitro* Kinetic Data to Predict Drug–Drug Interactions, 43
 - 2.4.1 Choice of Probe Substrates for Inhibition Studies, 43
 - 2.4.2 Determining the Mechanism of CYP450 Inhibition, 46

2.4.3 Prediction of <i>In Vivo</i> Drug–Drug Inhibition Interactions from <i>In Vitro</i> Data, 53	
2.5 Consideration of Non-CYP Enzymatic Systems, 58	
2.5.1 Flavin-Containing Monooxygenase (FMO), 58	
2.5.2 UDP-glucuronosyltransferase (UGT), 59	
2.5.3 Sulfotransferase (SULT), 61	
2.5.4 <i>N</i> -Acetyltransferase (NAT), 61	
2.5.5 Methyltransferase, 62	
2.5.6 Epoxidase Hydrolase, 62	
2.5.7 Aldehyde Oxidase and Dehydrogenase, 63	
2.5.8 Glutathione- <i>S</i> -transferase (GST), 63	
2.6 Summary, 63	
2.7 Acknowledgments, 64	
References, 64	
3 Inhibition of Drug-Metabolizing Enzymes and Drug–Drug Interactions in Drug Discovery and Development	75
<i>R. Scott Obach</i>	
3.1 Introduction, 76	
3.2 Laboratory Approaches Inhibiting Drug-Metabolizing Enzymes, 76	
3.2.1 Analytical Method, 77	
3.2.2 Determination of Linearity of Velocity, 77	
3.2.3 Substrate Saturation Experiment, 80	
3.2.4 Reversible Inhibition Experiments: K_i , 81	
3.2.5 Reversible Inhibition Experiments: IC_{50} , 84	
3.3 Selection of Substrates for Inhibition Experiments in Drug Metabolism, 85	
3.4 Inhibition of Drug-Metabolizing Enzymes in Drug Discovery and Development, 87	
3.4.1 Inhibition Experiments in Early Drug Discovery, 87	
3.4.2 Inhibition Experiments in Late Drug Discovery, 89	
3.4.3 Inhibition Experiments During Drug Development, 90	
3.5 Summary, 90	
References, 91	
4 Mechanism-Based CYP Inhibition: Enzyme Kinetics, Assays, and Prediction of Human Drug–Drug Interactions	95
<i>Magang Shou</i>	
4.1 Kinetic Model for Mechanism-Based Inhibition, 97	
4.2 Methodological Measurements of Kinetic Parameters, 99	
4.3 Incubation, 100	
4.3.1 CYP Isoform-Specific Assays, 100	
4.3.2 General Incubation Procedure and Sample Preparation, 100	

4.3.3 LC-MS-MS Analysis, 100	
4.3.4 Data Analysis, 102	
4.4 Prediction of Human DDIs from <i>In Vitro</i> MBI Data, 103	
4.5 Acknowledgments, 108	
References, 108	
5 Genomic Approaches To Drug-Drug Interactions	113
<i>Yi Yang and Jeffrey F. Waring</i>	
5.1 Introduction, 113	
5.2 DNA Microarrays, 114	
5.2.1 Array Platforms, 115	
5.2.2 Gene Expression Profiling Using Microarray, 115	
5.2.3 Genotyping Using Microarray, 117	
5.3 Genomic Application Toward the Prediction of DDIs, 117	
5.3.1 Gene Expression Profiling of Compound Mixtures, 118	
5.3.2 Expression Profiling of DMEs and Transporters, 118	
5.3.3 Identification of Gene Expression Patterns Indicative of DDIs, 120	
5.4 Genomics Approach to Decipher the Molecular Basis of DDI: Nuclear Receptors, 121	
5.5 Genomic Approaches to Address the Genetic Variability in DDIs, 122	
5.6 Conclusion, 124	
References, 124	
6 Transporters and Drug Interactions	131
<i>Yoshihisa Shitara, Toshiharu Horie, and Yuichi Sugiyama</i>	
6.1 Introduction, 131	
6.2 Interactions Involving Liver Transporters, 132	
6.2.1 Role of Transporters in the Biliary Excretion of Drugs, 132	
6.2.2 Transporter-Mediated DDIs in the Process of Hepatobiliary Excretion, 137	
6.2.3 Transporters as a Determinant of Metabolic Rate, 141	
6.3 Interactions in Intestine Transporters, 143	
6.3.1 Role of Transporters in Intestinal Absorption, 143	
6.3.2 Examples of Transporter-Mediated DDIs in the Process of Intestinal Absorption, 144	
6.4 Drug Toxicity Involving Drug Transporters, 148	
6.5 Drugs that Affect the Expression or Localization of Transporters, 149	
6.6 Conclusion, 151	
References, 151	

- 7 Transporter-Mediated Drug Interactions: Molecular Mechanisms and Clinical Implications** **159**
Jiunn H. Lin
- 7.1 Introduction, 159
 - 7.2 Tissue Distribution and Cellular Location of Transporters, 161
 - 7.2.1 Small Intestine, 161
 - 7.2.2 Liver, 165
 - 7.2.3 Kidney, 167
 - 7.2.4 Brain, 170
 - 7.3 Molecular Mechanisms for Transporter Inhibition and Induction, 172
 - 7.3.1 Inhibition of Transporters, 173
 - 7.3.2 Induction of Transporters, 174
 - 7.4 Drug Interactions Caused by Transporter Inhibition and Induction, 176
 - 7.4.1 Direct Evidence, 176
 - 7.4.2 Circumstantial Evidence, 178
 - 7.5 Clinical Significance of Transporter-Mediated Drug Interactions, 183
 - 7.6 Conclusion, 184
 - References, 185
- 8 Recent Case Studies of Clinically Significant Drug–Drug Interactions and the Limits of *In Vitro* Prediction Methodology** **195**
René H. Levy, Isabelle Ragueneau-Majlessi, and Carol Collins
- 8.1 Introduction, 195
 - 8.2 Case Studies, 196
 - 8.2.1 Interaction Between Repaglinide and Gemfibrozil + Itraconazole, 196
 - 8.2.2 Interaction Between Ramelteon and Fluvoxamine, 198
 - References, 199
- 9 U.S. Regulatory Perspective: Drug–Drug Interactions** **201**
John Strong and Shiew-Mei Huang
- 9.1 Introduction, 202
 - 9.2 An Integrated Approach, 202
 - 9.3 Methods for Evaluating Metabolic Clearance *In Vitro*, 204
 - 9.3.1 CYP Reaction Phenotyping, 204
 - 9.3.2 CYP Inhibition, 206
 - 9.3.3 CYP Induction, 208
 - 9.3.4 Other Metabolic Enzymes, 209
 - 9.3.5 Transporters, 209
 - 9.3.6 GLP Versus Non-GLP Studies, 210

9.4	<i>In Vivo</i> Approaches, 211	
9.4.1	Study Design, 211	
9.4.2	Data Analysis and Sample Size Consideration, 214	
9.4.3	Classification of Inhibitors and Labeling Considerations, 214	
9.4.4	Cocktail Approaches, 216	
9.4.5	P-gp and Other Transporters, 216	
9.5	Clinical Cases, 216	
9.6	Regulatory Considerations, 217	
9.7	Labeling, 219	
9.8	Summary, 220	
	References, 221	
10	Herbal Drug Interactions—A Canadian Perspective	227
	<i>Brian C. Foster</i>	
10.1	Introduction, 227	
10.2	Interaction Risk Determination, 229	
10.3	NHP Products, 231	
10.3.1	NHP Characterization, 232	
10.4	Disposition, 234	
10.5	PD and PK interactions, 235	
10.5.1	Choice of Substance Concentration Range, 236	
10.5.2	Role of Animal Studies, 236	
10.5.3	Human Clinical Studies, 237	
10.6	Action, 239	
	References, 240	
	Index	241
	Wiley Series in Drug Discovery and Development	244