

Contents

Preface *xiii*

List of Contributors *xv*

Part I The Academia – Market Bouncing of Peptide Drugs – Challenges and Strategies in Translational Research with Peptide Drugs 1

| | | |
|----------|--|----------|
| 1 | Peptides as Leads for Drug Discovery | 3 |
| | <i>Paul J. Edwards, and Steven R. LaPlante</i> | |
| 1.1 | Introduction | 3 |
| 1.2 | Overview of Process for Transforming Peptides to Peptidomimetics | 5 |
| 1.3 | HCMV Protease | 7 |
| 1.3.1 | HCMV Protease: Identification and Characterization of Antiviral Inhibitors Targeting the Serine Protease Domain of the Human Cytomegalovirus (HCMV Protease) | 7 |
| 1.3.2 | Mapping Essential Elements of the Substrate Peptides and Determining Structures of Ligands Bound to HCMV | 8 |
| 1.3.3 | Improving Peptide Activity to Allow SAR Studies | 10 |
| 1.3.4 | Elucidation of the Binding Mode of the Optimized Peptidyl Segment | 10 |
| 1.3.5 | Ligand Adaptations upon Binding | 12 |
| 1.3.6 | Strategic Summary for HCMV Peptide Mimic Design Process | 14 |
| 1.4 | HCV Protease | 15 |
| 1.4.1 | HCV Protease as an Antiviral Target | 15 |
| 1.4.2 | NS3 Serine Protease Possesses a Chymotrypsin-Like Fold | 16 |
| 1.4.3 | Discovery of the Peptide DDIVPC as an Inhibitor of NS3 Protease | 16 |
| 1.4.4 | “Sensemaking” and Knowledge Building: Mapping of the Critical Binding Residues of the Peptide and Creation of an Inhibitor-Protease Model | 18 |

Peptide Drug Discovery and Development: Translational Research in Academia and Industry, First Edition.
Edited by Miguel Castanho and Nuno C. Santos.
© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
Published 2011 by WILEY-VCH Verlag GmbH & Co. KGaA

| | | |
|----------|--|-----------|
| 1.4.5 | Knowledge Building: Monitoring Ligand Flexibility in the Free-State and Changes Upon Binding – P3 Rigidification | 18 |
| 1.4.6 | N-Terminal Truncation and Improved P1, P2 and P5 Substituents | 22 |
| 1.4.7 | Macrocyclization: Linking the Flexible P1 Side-Chain to P3 | 25 |
| 1.4.8 | HCV Protease Inhibitor BI00201335 | 29 |
| 1.5 | Herpes Simplex Virus | 32 |
| 1.5.1 | Herpes Simplex Virus-Encoded Ribonucleotide Reductase Inhibitors | 32 |
| 1.6 | Renin | 38 |
| 1.6.1 | Aspartyl Protease Renin as a Target | 38 |
| 1.7 | HIV | 45 |
| 1.7.1 | HIV Protease Inhibitors | 45 |
| 1.8 | Conclusions | 47 |
| 2 | Marketing Antimicrobial Peptides: A Critical Academic Point of View | 57 |
| | <i>Eduard Bardají</i> | |
| 2.1 | Introduction | 57 |
| 2.2 | Basic Research: Antimicrobial Peptides | 58 |
| 2.3 | Patents | 61 |
| 2.4 | Potential Applications of AMPs | 63 |
| 2.5 | Technology Transfer: Valorization, Licensing, or Spin-Off Creation | 64 |
| 2.6 | Spin-Off Creation: An Academic Point of View | 66 |
| 3 | Oral Peptide Drug Delivery: Strategies to Overcome Challenges | 71 |
| | <i>Hamman, Josias H. and Steenekamp, Jan H.</i> | |
| 3.1 | Introduction | 71 |
| 3.2 | Challenges Associated with Oral Peptide Delivery | 72 |
| 3.2.1 | Transport Pathways Across the Intestinal Epithelium | 72 |
| 3.2.2 | Unfavorable Physicochemical Properties of Peptide Drugs | 73 |
| 3.2.2.1 | Molecular Size, Hydrophilicity, and Physical Stability | 73 |
| 3.2.3 | Physical Barriers of the Gastrointestinal Tract | 73 |
| 3.2.3.1 | Transcellular Pathway | 73 |
| 3.2.3.2 | Paracellular Pathway | 75 |
| 3.2.4 | Biochemical Barriers of the Gastrointestinal Tract | 75 |
| 3.2.4.1 | Luminal Enzymes | 76 |
| 3.2.4.2 | Brush Border Membrane Bound Enzymes and Intracellular Enzymes | 76 |
| 3.2.5 | Efflux Transport Systems | 76 |
| 3.2.6 | Gastrointestinal Transit Time and Site-Specific Absorption | 77 |

| | | |
|----------|---|------------|
| 3.3 | Strategies to Overcome the Barriers of the Gastrointestinal Tract | 77 |
| 3.3.1 | Absorption Enhancing Agents | 77 |
| 3.3.2 | Chemical and Physical Modifications | 78 |
| 3.3.3 | Targeting Strategies | 81 |
| 3.3.3.1 | Targeting Specific Regions of the Gastrointestinal Tract | 81 |
| 3.3.3.2 | Targeting Receptors and Transporters | 82 |
| 3.3.4 | Formulation Strategies | 83 |
| 3.3.4.1 | Particulate Carrier Systems | 83 |
| 3.3.4.2 | Enzyme Inhibition | 84 |
| 3.3.4.3 | Mucoadhesive Systems | 84 |
| 3.4 | Conclusions | 84 |
| | | |
| 4 | Rational Design of Amphipathic α-Helical and Cyclic β-Sheet Antimicrobial Peptides: Specificity and Therapeutic Potential | 91 |
| | <i>Wendy J. Hartsock and Robert S. Hodges</i> | |
| 4.1 | Introduction to Antimicrobial Peptides | 91 |
| 4.2 | Antimicrobial and Hemolytic Activities of Amphipathic α -Helical Antimicrobial Peptides: Mechanisms and Selectivity | 92 |
| 4.3 | Structure–Activity Relationship Studies of Amphipathic α -Helical and Cyclic β -Sheet Antimicrobial Peptides: Optimization of Pathogen Selectivity and Prevention of Host Toxicity | 94 |
| 4.4 | Commercialization of Antimicrobial Peptides | 112 |
| 4.5 | Therapeutic Potential | 113 |
| | | |
| 5 | Conotoxin-Based Leads in Drug Design | 119 |
| | <i>Muharrem Akcan and David J. Craik</i> | |
| 5.1 | Introduction | 119 |
| 5.1.1 | Cone Snails | 119 |
| 5.1.2 | Conotoxin Discovery and Characterization (MS, cDNA, Peptide Sequencing) | 120 |
| 5.1.3 | Conotoxin Classification and Targets | 121 |
| 5.1.4 | Posttranslational Modifications (PTMs) | 122 |
| 5.1.5 | Prospects for Drug Discovery | 124 |
| 5.2 | Conotoxin Synthesis, Folding, and Structure | 124 |
| 5.2.1 | Synthesis | 124 |
| 5.2.2 | Folding | 127 |
| 5.2.3 | Structure by NMR and X-Ray | 127 |
| 5.3 | Conotoxins as Drug Leads | 128 |
| 5.3.1 | Overview of Conotoxins in Drug Design | 128 |
| 5.3.2 | ω -Conotoxins (MVIIA, CVII) D | 129 |
| 5.3.3 | α -Conotoxins (Vc1.1) | 129 |

- 5.3.4 χ -Conotoxins (MrIA) 130
- 5.3.5 Re-engineered Conotoxins in Drug Design 131
- 5.4 Conclusions 133

6 Plant Antimicrobial Peptides: From Basic Structures to Applied Research 139

Suzana M. Ribeiro, Simoni C. Dias, and Octavio L. Franco

- 6.1 Introduction 139
- 6.2 The Diversity of Plant Antimicrobial Peptides: Focusing on Tissue Localization and Plant Species Distribution 139
- 6.3 Possible Structural Folds Found in Plant AMPs to Date 140
- 6.4 New Biotechnological Products Produced from Plant Peptides 144

Part II Peptide Drugs' Translational Tales – Peptide Drugs Before, Through and After Industry Pipelines 157

7 Omiganan Pentahydrochloride: A Novel, Broad-Spectrum Antimicrobial Peptide for Topical Use 159

Evelina Rubinchik and Dominique Dugourd

- 7.1 Omiganan: A Novel Anti-Infective Agent for Topical Indications 159
- 7.2 Structure and Mechanism of Action 160
- 7.3 Spectrum of Activity 163
- 7.4 Preclinical Efficacy Studies 163
- 7.5 Preclinical Toxicology Studies 164
- 7.6 Clinical Studies 165
- 7.7 Conclusions 167

8 Turning Endogenous Peptides into New Analgesics: The Example of Kyotorphin Derivatives 171

Marta M.B. Ribeiro, Isa D. Serrano, and Sónia Sá Santos

- 8.1 Introduction 171
- 8.2 Peptides as Future Drug Candidates 171
- 8.3 Central Nervous System Analgesic Peptides 172
- 8.4 Endogenous Opioid System 173
- 8.5 Strategies to Deliver Analgesic Peptides to the Brain 174
- 8.6 Development of New Opioid-Derived Peptides 175
- 8.7 Kyotorphin – the Potential of an Endogenous Dipeptide 177
- 8.8 New KTP Derivatives 178
- 8.9 Assessing BBB Permeability with Peptide – Membrane Partition Studies 179

| | | |
|-----------|---|------------|
| 8.10 | Kyotorphins: Partition to the Membrane and Enhanced Analgesic Activity | 179 |
| 8.11 | Academia and Pharmaceutical Industry: Friends or Foes? | 183 |
| 9 | The Development of Romiplostim – a Therapeutic Peptibody Used to Stimulate Platelet Production | 189 |
| | <i>Graham Molineux and Ping Wei</i> | |
| 9.1 | Introduction | 189 |
| 9.2 | Thrombopoietin and c-Mpl | 189 |
| 9.3 | Discovery and Optimization of Romiplostim | 192 |
| 9.4 | Pharmacodynamics (PD) and Pharmacokinetics (PK) of Romiplostim | 194 |
| 9.5 | A Brief ITP Primer | 199 |
| 9.5.1 | Diagnosis and Treatment | 199 |
| 9.5.2 | Thrombopoietin and ITP | 200 |
| 9.6 | Romiplostim Clinical Data | 201 |
| 9.7 | Safety and Other Insights Gained from Romiplostim Design and Development | 203 |
| 10 | HIV vs. HIV: Turning HIV-Derived Peptides into Drugs | 209 |
| | <i>Henri G. Franquelim, Pedro M. Matos, and A. Salomé Veiga</i> | |
| 10.1 | Introduction | 209 |
| 10.2 | HIV-1 Envelope Protein | 209 |
| 10.3 | HIV Entry and Its Inhibition | 210 |
| 10.4 | HIV-1 Fusion Inhibitors: from Bench to Clinical Administration | 211 |
| 10.5 | New Strategies for Creating New HIV Fusion Inhibitor Peptides | 215 |
| 10.5.1 | Increasing Helicity and Binding to gp41 | 216 |
| 10.5.2 | Isomeric Peptides and Resistance to Proteolysis | 219 |
| 10.5.3 | Bacterially Expressed Peptides | 220 |
| 10.5.4 | Modification of Peptides by Derivatization with Lipids or Proteins | 220 |
| 10.6 | Drug-Resistance and Combination Therapy | 222 |
| 10.7 | Concluding Remarks | 223 |
| 11 | Sifuvirtide, A Novel HIV-1 Fusion Inhibitor | 231 |
| | <i>Xiaobin Zhang, Hao Wu, and Fengshan Wang</i> | |
| 11.1 | Ideal Drug Target HIV-1 gp41 | 231 |
| 11.2 | Structure-Based Drug Design of Sifuvirtide | 232 |
| 11.3 | High Potency of Sifuvirtide | 234 |
| 11.4 | Limited Drug Resistance | 235 |
| 11.5 | Enhancement of the Efficiency of Sifuvirtide by Biomembrane Selectivity | 236 |
| 11.6 | Pharmacokinetics of Sifuvirtide with Long Half-Life | 237 |

- 11.7 Stratification of Monotherapy 238
- 11.8 20 mg Sifuvirtide Once Daily vs. 100 mg T20 Twice Daily 239
- 11.9 Conclusions and Discussion 240

Part III Whither Peptide Drugs? Peptides Shaping the Future of Drug Development 245

12 Endogenous Peptides and Their Receptors as Drug Discovery Targets for the Treatment of Metabolic Disease 247

Mary Ann Pellemounter, Yuren Wang, and Ning Lee

- 12.1 Centrally Secreted Neuropeptide Systems 248
 - 12.1.1 Corticotropin Releasing Factor (CRF) Peptides 248
 - 12.1.2 Melanin Concentrating Hormone (MCH) 249
 - 12.1.3 Melanocortins 250
 - 12.1.4 Neuropeptide Y (NPY) 252
 - 12.1.5 Neuromedin U (NMU) and Neuromedin S (NMS) 254
 - 12.1.6 Opioids 255
 - 12.1.7 QRFP 256
- 12.2 Peripherally Secreted Neuropeptides 256
 - 12.2.1 Amylin 256
 - 12.2.2 Bombesin-Like Peptides (Bombesin and Gastrin-Releasing Peptide) 257
 - 12.2.3 Cholecystokinin (CCK) 258
 - 12.2.4 Ghrelin 259
 - 12.2.5 Glucagon-like Peptide-1 260
 - 12.2.6 Leptin 261
 - 12.2.7 Oxyntomodulin (OXM) 262
 - 12.2.8 PYY3-36 and PP 262
- 12.3 Summary 263

13 Translation of Motilin and Ghrelin Receptor Agonists into Drugs for Gastrointestinal Disorders 269

Gareth J. Sanger, John Broad, and David H. Alpers

- 13.1 Introduction 269
 - 13.1.1 Similarities and Differences Between Motilin and Ghrelin 269
 - 13.1.2 Clinical Potential of Motilin and Ghrelin Receptor Agonists 270
- 13.2 Motilin and Ghrelin Receptor Agonists Under Development 271
 - 13.3 Translational Value of Preclinical Assays 275
 - 13.3.1 Motilin 271
 - 13.3.1.1 Assays Relevant to the Therapeutic Mechanism of Action 271
 - 13.3.1.2 Assays Relevant to Possible Non-GI Activity 275
 - 13.3.2 Ghrelin 276
 - 13.3.2.1 Assays Relevant to the Therapeutic Mechanism of Action 276

| | | |
|-----------|---|------------|
| 13.3.2.2 | Assays Relevant to Non-GI Activity | 276 |
| 13.4 | Clinical Translation: Selecting the “Right” Patient Population | 277 |
| 13.4.1 | Critically Ill Patients with Delayed Gastric Emptying | 279 |
| 13.4.2 | Patients with Gastroparesis | 279 |
| 13.4.2.1 | Diabetic Gastroparesis | 281 |
| 13.4.2.2 | Parkinson’s Disease | 281 |
| 13.4.2.3 | Cyclic Nausea and Vomiting | 282 |
| 13.4.2.4 | Migraine | 282 |
| 13.4.2.5 | Functional Dyspepsia (FD) | 282 |
| 13.4.2.6 | Gastroesophageal Reflux Disease (GERD) | 283 |
| 13.4.2.7 | Anorexia and Decreased Appetite (Ghrelin Agonists Only) | 284 |
| 13.5 | Clinical Development of Motilin and Ghrelin Receptor Agonists | 284 |
| 13.6 | Conclusions | 285 |
| 14 | Of Mice and Men: Translational Research on Amylin Agonism | 295 |
| | <i>Jonathan D. Roth, Christine M. Mack, James L. Trevaskis, and David G. Parkes</i> | |
| 14.1 | Overview of Amylin Physiology | 295 |
| 14.2 | Pramlintide: An Amylin Agonist | 296 |
| 14.3 | Amylin Agonism: Translational Research in Insulin-Dependent Diabetes | 297 |
| 14.3.1 | Post-Prandial Hyperglucagonemia and Diabetes | 297 |
| 14.3.2 | Amylin Agonism and Glucagon: Preclinical and Clinical Studies | 297 |
| 14.3.3 | Gastric Emptying and Diabetes | 298 |
| 14.3.4 | Amylin Agonism and Gastric Emptying: Preclinical and Clinical Studies | 298 |
| 14.4 | Amylin Agonism: Translational Research in Obesity | 299 |
| 14.4.1 | Food Intake and Body Weight: Role of Endogenous Amylin | 299 |
| 14.4.2 | Food Intake and Body Weight: Pre-clinical Studies | 300 |
| 14.4.3 | Food Intake and Body Weight: Clinical Studies | 302 |
| 14.4.4 | Combination Studies | 304 |
| 14.4.5 | Amylin Agonism and Small Molecule Agents | 304 |
| 14.4.6 | Combined Amylin and Leptin Agonism | 305 |
| 14.4.7 | Future Areas for Amylin Agonism-Based Translational Research | 307 |
| 15 | Peptides and Polypeptides as Immunomodulators and Their Consequential Therapeutic Effect in Multiple Sclerosis and Other Autoimmune Diseases | 313 |
| | <i>Ruth Arnon, Michael Sela, and Rina Aharoni</i> | |
| 15.1 | Introduction | 313 |

| | | |
|--------|---|-----|
| 15.2 | Peptides as Antigens and Vaccines | 314 |
| 15.3 | Peptides as Immunomodulators | 315 |
| 15.4 | Development of Copolymer 1 – a Polypeptide Immunomodulator Drug for the Treatment of Multiple Sclerosis | 316 |
| 15.4.1 | Clinical Studies with Cop 1 in MS Patients | 317 |
| 15.4.2 | Immunological Mechanisms Involved in the Mitigation of Disease by Cop 1 | 318 |
| 15.4.3 | Immunomodulation by Cop 1 in the CNS | 320 |
| 15.4.4 | Neuroprotection and Augmentation of Neurotropic Factors in the Brain | 321 |
| 15.4.5 | Myelin Repair and Neurogenesis | 323 |
| 15.4.6 | The Effect of Cop 1 on Another Autoimmune Disease – Inflammatory Bowel Disease | 326 |
| 15.5 | Additional Immunomodulatory Peptides as Drug Candidates | 327 |
| 15.5.1 | Peptide Therapy for Type 1 Diabetes | 327 |
| 15.5.2 | Myasthenia Gravis (MG) | 328 |
| 15.5.3 | A Novel Tolerogenic Peptide for the Specific Treatment of Systemic Lupus Erythematosus | 328 |
| 15.6 | Summary and Concluding Remarks | 329 |

16 Development of Antibody Fragments for Therapeutic Applications

Sofia Côrte-Real, Frederico Aires da Silva, and João Gonçalves

| | | |
|----------|---|-----|
| 16.1 | Antibodies | 337 |
| 16.1.1 | Antibody Structure | 338 |
| 16.1.2 | Antibody Fragments | 341 |
| 16.1.3 | Single-Domain Antibodies | 343 |
| 16.1.4 | Engineering Multivalent, Bispecific, and Bifunctional Fragments | 345 |
| 16.1.5 | Intracellular Antibodies (Intrabodies) | 347 |
| 16.1.5.1 | Immunogenicity of Engineered Antibodies | 348 |
| 16.1.5.2 | Engineering New Protein Scaffolds | 349 |
| 16.2 | Conclusions | 350 |

Index 357