Diabetes and Its Complications:
Strategies to Advance Therapy and Optimize R&D
Allan B. Haberman, PhD
Executive Summary

Diabetes is a major disease characterized by hyperglycemia, or high blood glucose. This hyperglycemia results from the inability of the body to produce and/or to properly utilize insulin, the major hormone involved in glucose homeostasis. There are 2 major types of diabetes. Type 1 diabetes is a condition in which the body is unable to produce insulin due to autoimmune destruction of the insulin-secreting beta cells of the pancreas. It is treated principally by lifelong administration of injected insulin. Type 2 diabetes is a metabolic disease that results from a combination of insulin resistance (the inability of muscle, fat, and liver to respond efficiently to insulin) and pancreatic insufficiency (the inability of pancreatic beta cells to secrete enough insulin to compensate for the elevation of serum glucose levels caused by insulin resistance). It is treated principally by individualized combinations of diet and exercise, oral antidiabetic drugs, and insulin.

According to the World Health Organization, a worldwide epidemic of type 2 diabetes has been in progress since the mid-1980s. The worldwide number of diabetics was 30 million in 1985 and is projected to increase to at least 366 million by 2030. The vast majority of this increase is due to type 2 diabetes. The epidemic of type 2 diabetes is driven by increased rates of obesity, especially in industrialized countries and in emerging industrial countries such as India and China, coupled with the aging of the populations in both sets of countries.

In the United States, the American Diabetes Association (ADA) estimated that there were approximately 21 million people with diabetes in 2005, as well as 6.2 million individuals with undiagnosed diabetes. Diabetes was the sixth leading cause of death in the United States in that year.
Complications from Diabetes

Diabetes represents an enormous economic burden. The International Diabetes Federation states that diabetes accounts for 5% to 10% of health care expenditures in many countries throughout the world. The ADA estimated that the total cost of diabetes in the United States was $132 billion in 2002, including $92 billion in direct costs and $40 billion in indirect costs. The ADA expects this total cost to rise to $156 billion in 2010 and $192 billion in 2020. Most of the cost of diabetes is due to its complications, which result in cardiovascular disease, kidney failure, lower-extremity amputations, and blindness.

The American Association of Clinical Endocrinologists (AACE), in a report released at the association’s annual meeting in April 2007, estimated that the direct medical costs related to diabetes type 2 complications amounted to $22.9 billion in the United States in 2006. About 33% of people who have type 2 diabetes also have at least 1 of the other health problems associated with it, and many others have more than 1 complication, according to the AACE report, titled State of Diabetes Complications in America (www.stateofdiabetes.com).

Development of complications can be slowed or prevented in diabetics by maintaining tight blood glucose control, through the use of antidiabetic drugs (insulin and/or oral antidiabetic drugs). Prescription drugs represent only 13% of total diabetes costs. Therefore, the best strategy for controlling the costs of diabetes is diagnosis and adequate drug treatment to prevent development of complications. Development of novel drugs that address major unmet needs in diabetes is an important part of such a strategy.

This report is a strategic discussion of the field of drugs for diabetes and its complications, with an emphasis on novel and emerging drugs and therapeutic strategies. Chapter 1 provides the background for understanding the nature, epidemiology, and cost of diabetes. Chapter 2 discusses the genetics and pathobiology of type 1 diabetes and experimental therapeutic strategies for prevention of type 1 diabetes in susceptible individuals.

Chapter 3 is a discussion of the pathogenesis of type 2 diabetes and its relationship to obesity. This includes pathogenic pathways by which substances (such as adipokines, free fatty acids, and inflammatory mediators) secreted by adipocytes in obese individuals contribute to such conditions as insulin resistance in muscle, pancreatic beta-cell
response to blood glucose, and changes in liver physiology. Novel therapeutic strategies designed to deal with the role of inflammation in type 2 diabetes are also discussed, as is the role of genetic factors in development of beta-cell dysfunction.

Chapter 4 is a discussion of current diagnosis and treatment modalities for diabetes. This includes discussion of diet and exercise, insulin products used in treatment of type 1 and type 2 diabetes (both injected products and Pfizer’s recently approved inhaled insulin product Exubera), and established oral antidiabetics for treatment of type 2 diabetes. Also discussed is the 2006 American Diabetes Association /European Association for the Study of Diabetes consensus statement on an optimal protocol for using current antidiabetic drugs (oral antidiabetics and insulin) to treat type 2 diabetes.

Chapter 5 discusses novel classes of antidiabetics that include drugs introduced into the market in 2005 and 2006, as well as drugs in still newer classes that are now in corporate pipelines. The 3 drugs that were newly introduced in 2005/2006 include the amylin analog pramlintide (Amylin’s Symlin), the incretin mimetic exenatide (Amylin/Lilly’s Byetta), and the dipeptidyl peptidase-IV (DPP-IV) inhibitor sitagliptin (Merck’s Januvia). Other drugs belonging to these classes are in clinical development or preregistration, as are drugs belonging to other novel classes.

Chapter 6 is a discussion of leading research and preclinical stage drugs, and novel therapeutic strategies, for type 2 diabetes. The chapter also includes a discussion of current strategies to improve the effectiveness of drug discovery, especially as applied to type 2 diabetes, and strategies to more effectively move drugs from discovery to development with a greater probability of success, involving biomarkers, translational medicine, and proof-of-concept clinical trials. Most of the R&D programs discussed in Chapter 6 are biology-driven, and often emerged from basic research in academic laboratories. However, also included is a discussion of CytRx’s target identification program based on RNA interference screening, and programs in which medicinal chemistry and structure-based drug design have been used to discover compounds that modulate targets that were previously thought to be “undruggable.”

Chapter 7 discusses the pathobiology of diabetic complications, including a novel unifying model for induction of microvascular complications, and a novel model for induction of macrovascular complications in individuals with diabetes and insulin resistance. Also discussed are
strategies for prevention of diabetic complications and novel pipeline drugs for treatment of these complications. Novel therapeutic strategies for treatment of diabetic complications are also included.

Chapter 8 is a discussion of the outlook for new antidiabetic drugs.

**How to Meet the Unmet Needs?**

A key factor in the discovery and development of successful new antidiabetic drugs is addressing the major unmet needs in type 2 diabetes, especially the need for drugs that both lower blood glucose and enable patients to lose weight, and the need to slow or reverse the decline in pancreatic beta-cell function, which is the major cause of progression of the disease. The new incretin mimetic exenatide appears to meet the need for induction of weight loss to some extent, and there is evidence that both exenatide and DPP-IV inhibitors may slow or reverse decline in beta-cell function, although this evidence is not yet conclusive. Many of the early-stage drugs are designed to address these unmet needs. Other major unmet needs are the need for drugs to treat diabetic complications and to prevent the development of type 1 diabetes in susceptible individuals. Also needed are better strategies (whether based on diet and exercise or drug-based) to prevent type 2 diabetes in prediabetic individuals.

Economists estimate that the worldwide market for antidiabetic drugs was over $13 billion in 2004. Approximately 54% of this market was accounted for by oral antidiabetic drugs for type 2 diabetes, with the remainder due to insulin products. The market is expected to nearly double by 2009, with the growth largely driven by novel agents. Given the alarming worldwide growth in diabetes, and especially if companies are able to address major unmet needs, the outlook for the antidiabetic drug market is excellent.
Table of Contents

CHAPTER 1

INTRODUCTION ......................................................................................1

1.1. Risk Factors for Type 2 Diabetes ......................................................3
    Preventing Development of Diabetes in Prediabetic Individuals ......4

1.2. Growth in Prevalence of Diabetes ....................................................5
    Worldwide Increase in Obesity .........................................................6

1.3. Economic Burden of Diabetes ..........................................................7

1.4. Market Size for Current Diabetes Drugs .........................................8

1.5. Unmet Medical Needs in Diabetes ..................................................8
    Type 2 Diabetes ..............................................................................8
    Type 1 Diabetes .............................................................................11

CHAPTER 2

TYPE 1 DIABETES AS AN AUTOIMMUNE DISEASE ..................13

2.1. Genetic and Environmental Determination of
    Type 1 Diabetes .............................................................................13
    Genetic Determinants ....................................................................14
    Environmental Determinants .........................................................15

2.2. Pathogenesis of Type 1 Diabetes ..................................................16
    Trials of Agents to Prevent or Ameliorate Type 1 Diabetes
    in Patients with Prediabetes or New-Onset Diabetes .................18
    ENDIT and DPT-1 .......................................................................18
    Anti-CD3 Agents .........................................................................18
    New Approaches to Treatment of Established Type 1 Diabetes .....21
# CHAPTER 3

**TYPE 2 DIABETES AS A METABOLIC DISEASE**

## 3.1. Obesity as a Cause of Insulin Resistance and Beta-Cell Dysfunction
- Adipokines, Obesity, and Insulin Resistance ........................................25
- Free Fatty Acids as a Critical Factor in Both Insulin Resistance and Beta-Cell Dysfunction .................................................................28
- Obesity, Inflammation, and Insulin Resistance .....................................31
  - Salicylates as Therapeutic Drugs ...................................................33
  - Chemical Chaperones as Therapeutic Agents: PBA and TUDCA .................................................................35

## 3.2. Genetic Factors in Development of Beta-Cell Dysfunction
- Activating Mutations in the KCNJ11 Gene .......................................38
- TCF7L2: A Major Risk Factor for Late-Onset Type 2 Diabetes ..........39
- Type 2 Diabetes Is Caused by a Combination of Genetic Risk Factors .................................................................41

# CHAPTER 4

**CURRENT DIAGNOSIS AND TREATMENT OF DIABETES**

## 4.1. Diagnosis of Diabetes .................................................................43

## 4.2. Treatment of Diabetes .................................................................45
- Diet and Exercise ................................................................................45
- Concurrent Treatment of Other Aspects of the Metabolic Syndrome .................................................................46

## 4.3. Insulin Products ........................................................................46
- Insulin Formulations with Different Durations of Action ...............47
  - Long-Acting Insulin Glargine .........................................................48
  - Long-Acting Insulin Detemir .........................................................48
- Inhaled Insulin ....................................................................................48

## 4.4. Established Oral Antidiabetics ..................................................49
- Sulfonylureas .....................................................................................51
- Biguanides: Metformin .....................................................................51
- Meglitinides ....................................................................................53
- Alpha-glucosidase Inhibitors ..............................................................53
- Thiazolidinediones ............................................................................54

## 4.5. Type 2 Diabetes Management Using the Established Oral Antidiabetics and Insulin .................................................................54
- ADA/EASD Consensus Statement .....................................................55
- ADA/EASD Panel's Recommendations versus Traditional Treatments .................................................................57
- De-emphasis of Newer Drugs in the Panel's Recommendations ....57
- Disagreement with the Panel's Findings ...........................................59
CHAPTER 5

NOVEL AND EMERGING ANTIDIABETIC DRUGS ...............61

5.1. Approved and Pipeline Drugs Belonging to Drug Classes

Introduced into the Market since 2005 ........................................62
Amylin Analogs: Pramlintide ............................................................63
Incretin Mimetics...........................................................................64
Exenatide ....................................................................................64
GSK716155................................................................................66
Liraglutide ....................................................................................66
Dipeptidyl Peptidase-IV Inhibitors ....................................................66
Sitagliptin ....................................................................................67
Vildagliptin ..................................................................................68
Saxagliptin ..................................................................................68
PSN9301 ......................................................................................68

Outlook for the Newly Introduced Antidiabetic Drugs ..........69

5.2. Novel Classes of Antidiabetics in Corporate Pipelines ..........70

PPARα/PPARγ Dual Agonists (Glitazars) ..............................72
PPARγ Partial Agonists ...............................................................73
MBX-102 ......................................................................................74
FK614 and PA-082 ......................................................................76
PPARα, β, γ Pan-Agonists .............................................................76
Cannabinoid-1 Receptor Antagonists ...........................................77
RIO-Diabetes Study ..................................................................78
SERENADE ................................................................................79

11 Beta-hydroxysteroid Dehydrogenase Type 1 .......................80
Sodium Glucose Cotransporter-2 Inhibitors ..............................82
Glucokinase Activators ...............................................................82

CHAPTER 6

EARLY-STAGE DRUGS AND NOVEL THERAPEUTIC
STRATEGIES FOR TYPE 2 DIABETES .................................85

6.1. Does the Lack of Scientific Knowledge of Type 2 Diabetes
Hamper Development of Effective Treatments? .......................86

6.2. The Inadequacy of Animal Models in Type 2 Diabetes ..........87

6.3. Strategies for Making Drug Discovery and Development
More Effective .............................................................................89
Dealing with Multiple Molecular “Causes” of Disease by
Hitting More than One Target ......................................................91
Whole-Pathway Approaches .........................................................92
Biology-Driven Drug Discovery ....................................................92
Biomarkers and Translational Medicine ....................................93
Animal Models and Complex Diseases .....................................96
<table>
<thead>
<tr>
<th>Chapter 6</th>
<th>Table of Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.4. The CHI Diabetes Survey and Issues Related to Strategies to Improve the Effectiveness of Drug Discovery and Development</td>
<td>96</td>
</tr>
<tr>
<td>6.5. Selected Research-Stage Agents and Novel Therapeutic Strategies for Type 2 Diabetes</td>
<td>99</td>
</tr>
<tr>
<td>Small-Molecule GLP-1 Receptor Agonists</td>
<td>101</td>
</tr>
<tr>
<td>Boc5</td>
<td>103</td>
</tr>
<tr>
<td>Ago-Allosteric Modulators</td>
<td>103</td>
</tr>
<tr>
<td>Protein Tyrosine Phosphatase 1B Inhibitors</td>
<td>104</td>
</tr>
<tr>
<td>Premclinical Development of Small-Molecule PTP1B Inhibitors</td>
<td>105</td>
</tr>
<tr>
<td>Development of an Antisense PTP1B Inhibitor: ISIS 113715</td>
<td>106</td>
</tr>
<tr>
<td>AMP-Activated Protein Kinase Activators</td>
<td>107</td>
</tr>
<tr>
<td>CytrRx Corporation’s RNAi-Based Drug Discovery Programs in Type 2 Diabetes and Obesity</td>
<td>110</td>
</tr>
<tr>
<td>Receptor-Interacting Protein 140</td>
<td>111</td>
</tr>
<tr>
<td>MAP4K4</td>
<td>112</td>
</tr>
<tr>
<td>Sirtuin Modulators</td>
<td>113</td>
</tr>
<tr>
<td>Ghrelin Antagonists (Growth Hormone Secretagogue Receptor Antagonists)</td>
<td>119</td>
</tr>
<tr>
<td>GPR119 Agonists</td>
<td>121</td>
</tr>
<tr>
<td>6.6. Conclusions: Development of Novel Antidiabetics and the CHI Diabetes Survey</td>
<td>121</td>
</tr>
</tbody>
</table>

**CHAPTER 7**

<table>
<thead>
<tr>
<th>Chapter 7</th>
<th>DIABETIC COMPLICATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1. Microvascular Complications</td>
<td>126</td>
</tr>
<tr>
<td>Diabetic Retinopathy</td>
<td>126</td>
</tr>
<tr>
<td>Diabetic Neuropathy</td>
<td>127</td>
</tr>
<tr>
<td>Diabetic Nephropathy</td>
<td>128</td>
</tr>
<tr>
<td>7.2. Prevention of Diabetic Complications</td>
<td>128</td>
</tr>
<tr>
<td>7.3. Pathogenesis of Diabetic Complications</td>
<td>131</td>
</tr>
<tr>
<td>Four Pathogenic Pathways in Diabetic Complications</td>
<td>131</td>
</tr>
<tr>
<td>A Unifying Model for Induction of Microvascular Diabetic Complications</td>
<td>134</td>
</tr>
<tr>
<td>Induction of Macrovascular Complications in Insulin-Resistant and Diabetic Individuals</td>
<td>137</td>
</tr>
<tr>
<td>7.4. Novel Drugs and Therapeutic Strategies for Diabetic Complications</td>
<td>138</td>
</tr>
<tr>
<td>Drugs for Diabetic Complications in Clinical Trials</td>
<td>138</td>
</tr>
<tr>
<td>Ranirestat</td>
<td>140</td>
</tr>
<tr>
<td>Ranibizumab and Pegaptanib</td>
<td>140</td>
</tr>
<tr>
<td>Ruboxistaurin</td>
<td>140</td>
</tr>
<tr>
<td>Alagebrum</td>
<td>141</td>
</tr>
<tr>
<td>Pyridoxamine</td>
<td>144</td>
</tr>
<tr>
<td>Sulodexide</td>
<td>144</td>
</tr>
</tbody>
</table>
7.5. Novel Therapeutic Strategies for Diabetic Complications ..........145
   Therapeutic Strategies Based on Brownlee’s Unified
   Diabetic Complications Model ..........................................................145
     Development of Transketolase Activators ................................145
     Development of PARP Inhibitors .............................................146
     Development of Catalytic Antioxidants ..............................146
   A Novel Therapeutic Strategy for Diabetic Retinopathy Based
   on Targeting Extracellular Carbonic Anhydrase and Kallikrein ....147

CHAPTER 8

OUTLOOK ..............................................................................................149
8.1. A Disease of Progress .................................................................149
8.2. Where to Go from Here ...............................................................151
8.3. Addressing Unmet Needs ..............................................................152
8.4. Aiming for Multiple Targets .........................................................153

APPENDIX

INSIGHT PHARMA REPORTS – DIABETES SURVEY –
   JANUARY 2007 ..........................................................155

References ............................................................................................167
Glossary of Selected Terms .................................................................185
Company Index with Web Addresses ..............................................187

TABLES
Table 1.1. Major Social and Economic Factors that Drive the Worldwide
   Increase in Obesity ..............................................................................7
Table 3.1. Selected Genetic Factors Implicated in Development of
   Beta-Cell Dysfunction in Type 2 Diabetes ........................................37
Table 4.1. Established Classes of Oral Drugs for Treatment of
   Type 2 Diabetes ..............................................................................50
Table 5.1. Classes of Antidiabetic Drugs Introduced into the Market
   since 2005 .......................................................................................62
Table 5.2. Novel Classes of Antidiabetic Drugs Now in Clinical Trials ....71
Table 6.1. Selected Research-Stage Agents and Novel Therapeutic
   Strategies for Type 2 Diabetes ..........................................................100
Table 7.1. Selected Pipeline Drugs for Diabetic Complications ..........139
FIGURES

Figure 1.1. Projected Growth in Worldwide Prevalence of Diabetes, 1985 to 2030 .......................................................... 5
Figure 3.1. Insulin-Signaling Pathway ........................................ 27
Figure 5.1. DPP-IV Inhibitors versus TZDs .................................... 70
Figure 6.1. CHI Survey: Involvement in Biomarker Discovery .......... 97
Figure 6.2. CHI Survey: Importance of Biomarkers in Clinical Trials .. 98
Figure 6.3. CHI Survey: Importance of Collaboration in Business Plans .. 99
Figure 6.5. CHI Survey: Focus of Respondents ............................ 122
Figure 6.6. CHI Survey: Focus of Type 2 Drugs in Development ............ 123
Figure 7.1. Unified Diabetic Complications Model ......................... 136

SURVEY FIGURES

Figure 1A. Response by Sector ............................................. 155
Figure 2A. Focus of Respondents ............................................ 156
Figure 3A. Changes in Levels of Involvement ............................ 157
Figure 4A. Focus of Increased Activities .................................. 157
Figure 5A. Type 2 Diabetes Drugs to Launch 2007-2008 ............... 158
Figure 6A. Type 2 Diabetes Drugs in Pipelines .......................... 159
Figure 7A. Diabetes Complications Drugs in Pipelines .................. 159
Figure 8A. Obesity Drugs in Pipelines .................................... 160
Figure 9A. Antiobesity Drugs vs. Type 2 Diabetes Drugs ............... 161
Figure 10A. Scientific Knowledge vs. Drug Development ............. 161
Figure 11A. Focus of Type 2 Drugs in Development ................... 162
Figure 12A. Focus of Diabetes Complications Drugs in Development .. 163
Figure 13A. Involvement in Biomarker Discovery ....................... 163
Figure 14A. Importance of Biomarkers in Clinical Trials ............... 164
Figure 15A. Importance of Collaboration in Business Plans .............. 164
Figure 16A. Primary Modes of Collaboration ............................. 165
Figure 17A. DPP-IV Inhibitors vs. TZDs ................................. 166
hexosamine pathway by azaserine or genetic modification of genes for the targets of the hexosamine pathway blocked induction of these changes.\textsuperscript{150} These experiments thus demonstrated the importance of the hexosamine pathway in diabetic complications.

A Unifying Model for Induction of Microvascular Diabetic Complications

Dr. Brownlee has proposed, and obtained evidence for, a unifying model for diabetic complications.\textsuperscript{150} This model provides a means of linking the 4 pathogenic pathways discussed earlier via a common mechanism. It also provides for new therapeutic strategies for diabetic complications. Because of the generally disappointing clinical results with drugs discovered based on single pathogenic pathways, new therapeutic strategies are needed.

Dr. Brownlee and his colleagues compared biochemical processes in cells that cannot downregulate glucose transport under conditions of hyperglycemia (i.e., the cell types that are damaged by hyperglycemia, leading to diabetic complications) versus cell types that can downregulate glucose transport and thus remain undamaged by hyperglycemia. These researchers found that what consistently differentiated hyperglycemia-susceptible cells was increased production of ROS; they then found that the ROS originated in the mitochondria. In cells with a high intracellular glucose concentration, an excess of pyruvate is oxidized in the TCA cycle. This can overload electron transport in the mitochondria, resulting in its blockage. A key component of the mitochondrial electron transport chain, coenzyme Q, donates electrons one at a time to molecular oxygen, resulting in the formation of the ROS superoxide. Superoxide dismutase then converts superoxide to hydrogen peroxide ($\text{H}_2\text{O}_2$), another ROS. Moreover, in hyperglycemic cells, mitochondrial proteins can become glycated, which inhibits mitochondrial function and results in formation of excess superoxide.\textsuperscript{152}

The researchers demonstrated that the above sequence of events was true by a number of experiments. For example, when manganese superoxide dismutase (MnSOD) is genetically overexpressed, hyperglycemia does not increase ROS. This demonstrates that the initial ROS species formed under conditions of intracellular
hyperglycemia is superoxide. When uncoupling protein-1 (UCP-1) is overexpressed, hyperglycemia again does not increase ROS. UCPs are proteins that uncouple the process by which mitochondria convert electrical energy generated in the mitochondria by oxidation of catabolic products of glucose to ATP, and release the energy as heat. Overexpression of UCP-1 results in reversal of mitochondrial overload that results from hyperglycemia, and thus prevents production of superoxide.

In another set of experiments, the researchers produced endothelial cells that were depleted in mitochondrial DNA and thus had no functional mitochondria. Hyperglycemia failed to induce production of ROS in these cells and also failed to activate the polyol pathway, AGE formation, the PKC pathway, or the hexosamine pathway in these cells that lacked functional mitochondria. Moreover, overexpression of either MnSOD or UCP-1 also knocks out induction of all 4 pathogenic pathways by intracellular hyperglycemia. These studies indicate that hyperglycemia activates all 4 pathogenic pathways via inducing ROS in the mitochondria of susceptible cells.

Studies by the Brownlee group also indicate that in hyperglycemia-susceptible cells, superoxide activates the 4 pathogenic pathways by inhibiting a key glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Inhibition of GAPDH in cells with high intracellular levels of glucose does not occur when superoxide production is prevented by overexpression of MnSOD or UCP-1, or by inhibition of mitochondrial activity by specific chemical inhibitors.153

When GAPDH activity is inhibited, the levels of all glycolytic intermediates upstream from this enzyme are increased. This results in activation of all 4 pathogenic pathways, as illustrated in Figure 7.1.150
In cells with a high intracellular concentration of glucose, mitochondrial function is perturbed, resulting in production of excess levels of superoxide. This results in induction of PARP, which inhibits the enzyme GAPDH. As a result, glycolytic intermediates upstream from GAPDH are increased. Increases in glucose and 2 of the glycolytic intermediates activate all 4 pathogenic pathways that cause microvascular diabetic complications.

AGE, advanced glycation end product; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PARP, poly (ADP ribose) polymerase; PKC, protein kinase C; TCA cycle, tricarboxylic acid cycle.

*In cells with a high intracellular concentration of glucose, mitochondrial function is perturbed, resulting in production of excess levels of superoxide. This results in induction of PARP, which inhibits the enzyme GAPDH. As a result, glycolytic intermediates upstream from GAPDH are increased. Increases in glucose and 2 of the glycolytic intermediates activate all 4 pathogenic pathways that cause microvascular diabetic complications.

AGE, advanced glycation end product; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PARP, poly (ADP ribose) polymerase; PKC, protein kinase C; TCA cycle, tricarboxylic acid cycle.

**Source:** Haberman Associates

In further studies, Dr. Brownlee and his colleagues found that ROS generated in cells with high intracellular levels of glucose inhibits GAPDH via inducing poly (ADP ribose) polymerase (PARP). PARP is a DNA repair enzyme that exists in an inactive form in the nucleus. ROS causes single-strand breaks in DNA, resulting in the activation of PARP. PARP creates polymers of ADP-ribose, which it obtains by
hydrolysis of the cofactor nicotinamide adenine dinucleotide (NAD\(^{+}\)), a cofactor that is also involved in the glycolytic pathway (see Figure 7.1). The function of PARP is to recruit other DNA repair enzymes so that single-strand breaks in DNA may be repaired. However, activated PARP may attach polymers of ADP-ribose to GAPDH, thus inactivating it. GAPDH, which plays its well-known role in glycolysis, is a multifunctional enzyme that has additional functions in the nucleus and in cell membranes. In the case of cells with high intracellular levels of glucose, poly (ADP-ribose)ation of GAPDH results in the activation of the 4 pathogenic pathways associated with diabetic complications. Overexpression of either UCP-1 or MnSOD prevents this from happening by blocking ROS formation. Chemical PARP inhibitors also block activation of the pathogenic pathways by inhibiting poly (ADP-ribose)ation of GAPDH.\(^{150,154}\)

Novel therapeutic strategies based on this unified model are discussed later in this chapter.

**Induction of Macrovascular Complications in Insulin-Resistant and Diabetic Individuals**

Dr. Brownlee and his colleagues have developed a model for mechanisms of induction of macrovascular complications of insulin resistance, which is based on related pathways to the unified model for microvascular diabetic complications.\(^{150,155}\) In this model, the increased flux of free fatty acids (FFAs) in arterial endothelial cells in insulin resistance results in a similar perturbation in mitochondrial function to that seen in microvascular endothelial cells with an increased flux of glucose, as discussed in the section on a unified model for induction of microvascular complications. As a result, superoxide is overproduced in arterial endothelial cells subject to an increased flux of FFAs. The experimental basis for this model consists of studies in cell culture and in animal models.

In these cellular and animal models, FFAs activate pathogenic pathways of diabetic complications in arterial (macrovascular) cells, but not in microvascular endothelial cells as with glucose. FFA-induced ROS inhibits GAPDH, activates PKC and the hexosamine pathway, and induces AGE formation. As with GAPDH inhibition and pathogenic pathway activation by glucose in microvascular endothelial cells, all these activities are prevented by overexpression of UCP-1 or MnSOD. They are also blocked by inhibition of carnitine palmitoyltransferase type I (CPT-I), the rate-limiting enzyme for mitochondrial FFA oxidation.
FFA-induced ROS in arterial endothelial cells also inactivates key anti-atherogenic enzymes—prostacyclin synthase and endothelial nitric oxide synthase (eNOS). In arterial cells, eNOS is responsible for production of nitric oxide (NO), a potent vasodilator that helps maintain normal endothelial function and prevent the dysfunctional state associated with atherogenesis. NO also prevents platelet aggregation and smooth muscle cell proliferation, which are also involved in atherogenesis. Prostacyclin has similar activities that are complementary to those of NO. FFA-induced inhibition of prostacyclin synthase and eNOS was also completely prevented by inhibition of CPT-I or overexpression of UCP-1 or MnSOD, consistent with the role of FFA oxidation in the mitochondria and the resulting production of superoxide in the inhibition of the 2 enzymes.

The above model appears to account, at least in part, for the increased risk of atherosclerotic CVD in insulin-resistant individuals. However, adipokines and inflammatory mediators produced by adipocytes in obesity (discussed in Chapter 3) may also play a role. Moreover, diabetics have an even greater risk of CVD than do nondiabetic insulin-resistant individuals. Dr. Brownlee and his colleagues hypothesize that the effect of mitochondrial oxidation of glucose and of FFA on overproduction of superoxide in arterial endothelial cells of diabetics may be additive. This may account, at least in part, for the high risk of CVD in diabetics.

7.4. Novel Drugs and Therapeutic Strategies for Diabetic Complications

Drugs for Diabetic Complications in Clinical Trials

As discussed earlier in this chapter, treatments for diabetic complications are inadequate, and the best strategy for dealing with them is prevention by maintaining tight serum glucose levels or preventing diabetes altogether. However, especially with the epidemic of type 2 diabetes, the prevalence of diabetic complications is growing, and treatments for them thus constitute a major unmet medical need.

Strategies based on 3 of the 4 pathogenic pathways for diabetic complications, as well as some of the consequences of these complications such as vascular leak and angiogenesis in diabetic retinopathy and structural changes in the diabetic kidney, have resulted in the discovery of several drugs that are now in clinical trials. (See Table 7.1 for a selected list.)
As discussed earlier, many companies have attempted to develop aldose reductase inhibitors (ARIs), which inhibit the central enzyme of the polyol pathway. However, these compounds generally gave disappointing results in clinical trials. For example, in 2000, Pfizer suspended development of the ARI zenarestat, because of potential renal toxicity seen in Phase III clinical trials in diabetic neuropathy. This was despite the beneficial effects in nerve conduction velocity seen in the Phase III trial and in the previous Phase II study. Other agents gave disappointing efficacy results as well.

### Table 7.1. Selected Pipeline Drugs for Diabetic Complications

<table>
<thead>
<tr>
<th>Drug</th>
<th>Company</th>
<th>Stage</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranirestat</td>
<td>Sumitomo/Kyorin/Eisai</td>
<td>Phase III, neuropathy</td>
<td>Oral aldose reductase inhibitor</td>
</tr>
<tr>
<td>Ranibizumab (Lucentis)</td>
<td>Genentech</td>
<td>Phase II, retinopathy</td>
<td>Anti-VEGF MAb, administered by intravitreous injection; marketed for treatment of neovascular age-related macular degeneration</td>
</tr>
<tr>
<td>Pegaptanib (Macugen)</td>
<td>OSI Pharmaceuticals/Pfizer (originally developed by Eyetech, which OSI acquired in 2005)</td>
<td>Phase II, retinopathy</td>
<td>Anti-VEGF MAb, administered by intravitreous injection; marketed for treatment of neovascular age-related macular degeneration</td>
</tr>
<tr>
<td>Ruboxistaurin (Arxxant)</td>
<td>Lilly</td>
<td>Preregistration, retinopathy; received Approvable Letter from FDA in August 2006</td>
<td>Oral PKC beta inhibitor</td>
</tr>
<tr>
<td>Alagebrium</td>
<td>Alteon</td>
<td>Phase II, nephropathy</td>
<td>Oral AGE cross-link breaker; also in Phase II trials in diastolic heart failure, which is the product's main indication; diastolic heart failure is especially common in diabetics</td>
</tr>
<tr>
<td>Pyridoxamine (Pyridor)</td>
<td>NephroGenex (licensed from BioStratum in 2006)</td>
<td>Phase II, nephropathy</td>
<td>Oral inhibitor of AGE formation and scavenger of ROS; also being developed for acute renal failure</td>
</tr>
<tr>
<td>Sulodexide (Sulonex)</td>
<td>Keryx Biopharmaceuticals</td>
<td>Phase III/IV nephropathy</td>
<td>Oral glycosaminoglycan (80% fast-moving heparin and 20% dermatan sulfate)</td>
</tr>
</tbody>
</table>

AGE, advanced glycation end product; MAb, monoclonal antibody; PKC, protein kinase C; VEGF, vascular endothelial growth factor.

**Source: Haberman Associates**
Ranirestat

One agent, Sumitomo Pharma/Kyorin/Eisai’s ranirestat, is in Phase III clinical trials in diabetic neuropathy. In a published double-blind placebo-controlled Phase II study, ranirestat gave potent polyol pathway inhibition (as shown by a strong reduction of nerve sorbitol levels upon biopsy) and improved sensory nerve conduction velocities in patients with mild to moderate sensorimotor diabetic neuropathy over a 12-week period. Patients who completed the initial trial were offered an additional 48-week open-label treatment with the drug. Ranirestat showed continued polyol pathway inhibition and improvements in nerve conduction velocities, as well as improvements in signs and symptoms of diabetic neuropathy, over the longer period. The drug was well tolerated.156

Ranibizumab and Pegaptanib

Two monoclonal antibody (MAb) agents have recently been developed for vascular diseases of the eye—ranibizumab (Genentech’s Lucentis) and pegaptanib (OSI/Pfizer’s Macugen, originally developed by Eyetech, which was acquired by OSI in 2005). Both of these MAbs target VEGF, a growth factor that is involved in the vascular leakage and angiogenesis that characterize diabetic retinopathy. [The development of Lucentis was a byproduct of the development of an earlier anti-VEGF MAb drug, bevacizumab (Avastin), which is approved for certain cancer indications. Lucentis is an antibody fragment in a formulation that is optimized for ophthalmic use, as compared to Avastin, which is delivered systemically.] Both Lucentis and Macugen are approved for use in age-related macular degeneration, which has similar issues of vascular leak and angiogenesis, mediated by VEGF. Both drugs are in Phase II clinical trials in diabetic retinopathy. Lucentis and Macugen are both delivered by intravitreous injection into the eye, performed by ophthalmologists.

Ruboxistaurin

Lilly is developing an oral, small-molecule agent for diabetic retinopathy, ruboxistaurin (Arxxant), which is now in preregistration with the FDA. Ruboxistaurin is an inhibitor of PKC beta and thus targets the PKC pathway of diabetic complications.

Researchers published the results of a 36-month Phase III clinical trial of ruboxistaurin in December 2006.157 The study involved 685 patients with nonproliferative retinopathy at 70 clinical sites and was a randomized double-masked placebo-controlled trial. The trial showed a
statistically significant reduction in vision loss, need for laser treatment, and progression of macular edema, with an increased occurrence of visual improvement as compared to placebo. For example, sustained moderate vision loss occurred in 5.5% of ruboxistaurin-treated patients, as compared to 9.1% of placebo-treated individuals. According to Lilly, pooled data from two 3-year Phase III clinical trials with 813 patients with moderate to severe nonproliferative retinopathy showed that ruboxistaurin reduced the risk of sustained moderate vision loss by 41% as compared to placebo. Vision loss occurred in 6.1% of drug-treated patients, as compared to 10.2% of placebo-treated individuals.

In August 2006, the FDA issued an Approvable Letter for ruboxistaurin requesting additional clinical efficacy data. Lilly is appealing this decision, and additional clinical trials with ruboxistaurin are ongoing.

**Alagebrium**

Alteon has been developing its lead drug, alagebrium, for diabetic complications and diseases of aging. Alagebrium is an orally available AGE cross-link breaker and thus targets the AGE pathway of diabetic complications.

As of September 2006, the new management of Alteon made the strategic decision to focus the company’s efforts on the development of alagebrium for diastolic heart failure. The results of 2 Phase II clinical trials indicate that alagebrium is a promising agent for this life-threatening macrovascular condition, the prevalence of which is growing rapidly. It is especially seen in older patients, women, and diabetics. Diastolic heart failure may thus represent an additional macrovascular diabetic complication.

Diastolic dysfunction is seen in approximately 40% of diabetics, and it correlates with poor glycemic control. Although researchers have advanced several hypotheses to explain diastolic dysfunction and diastolic heart failure in diabetics, Alteon researchers hypothesize that it is caused by AGE cross-linking of structural proteins such as collagen and elastin, resulting in stiffening of large arteries. Alteon’s Phase II studies of alagebrium, which have shown efficacy in treatment of diastolic heart failure, are consistent with that hypothesis.

In July 2006, Alteon announced that one of its academic clinical collaborators had been awarded a grant from the Juvenile Diabetes Research Foundation to help fund a multinational Phase II trial of