Highlights from the Society for Medicines Research symposium
Type II Diabetes: Mechanisms and Emerging Therapeutic Targets,
held June 17, 2004, in London, United Kingdom.

Meeting the Needs of Type 2 Diabetes Patients

by Robert Williams and Ian Morris

Summary

The Society for Medicines Research symposium *Type II Diabetes: Mechanisms and Emerging Therapeutic Targets* was held June 17, 2004, at the National Heart and Lung Institute, Imperial College, London, United Kingdom. The conference brought together an international program of speakers representing academia, small biotech companies and large pharmaceutical companies to review approaches aimed at increasing our understanding of the etiology of type 2 diabetes and advances in the development of novel therapeutics. Type 2 diabetes is a major, worldwide healthcare problem, and the incidence of this disease is rising. In the United States alone, 13.3 million people were diagnosed with diabetes in 2002, an increase of 5.8 million in a decade following an alarming trend beginning in the 1980s. People who have diabetes are at an increased risk of developing serious life-threatening complications, notably cardiovascular disease, as well as experiencing morbidity, which severely impairs their quality of life. This trend will pose an increasing burden on governmental healthcare budgets.

It is not often that the start of a meeting confronts the audience with a cryptic clue of what is to come next in a presentation, but that is just what the first speaker of the Society for Medicines Research symposium *Type II Diabetes: Mechanisms and Emerging Therapeutic Targets*, held June 17, 2004, in London, United Kingdom, did. Victor Zammit from the Hannah Research Institute (Scotland, U.K.) presented his first slide, which said *What if Minowski had been ageusic?*, to the bewilderment of some of the audience. It, however, soon became obvious that Dr. Zammit was presenting the essential role of fatty acid metabolism in the etiology of type 2 diabetes, initially by drawing attention to the article published in *Science* by John McGarry (McGarry, J. *What if Minowski had been ageusic? An alternative angle on diabetes.* Science 1992, 285: 766–70).

Diabetes has long been considered a disease of glucose metabolism, but it has become clear in recent years that it is also a disease of deranged lipid metabolism. Metabolic syndrome, first defined in 1989, is a condition that predisposes to the development of type 2 diabetes and is a collection of health risks including abdominal obesity, high serum triglycerides, low high-density lipoprotein cholesterol, high blood pressure and elevated blood glucose. This condition has also been referred to as insulin resistance syndrome, as decreased efficacy of insulin is an important characteristic. Fatty acids affect glucose metabolism, insulin signaling and insulin secretion at multiple levels. For instance, a key finding has been that malonyl-CoA, a product of glucose metabolism, inhibits fatty acid oxidation via inhibition of carnitine palmitoyltransferase. This leads to increased synthesis of di- and triglycerides, which in turn inhibit insulin-mediated glycogen synthesis and glucose uptake. Elevated malonyl-CoA levels have also been implicated in dysregulation of satiety signaling. Dr. Zammit also was able to link pancreatic β-cell dysfunction in the prediabetic state to free fatty acid exposure through

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downregulation of the peroxisome proliferator-activated receptor γ (PPARγ). The emergence of insulin resistance and β-cell dysfunction occurs in parallel, in spite of having a different mechanistic origin. Thus, the multiple effects of free fatty acids and glucose synergize in the etiology of type 2 diabetes, offering the prospect of a diversity of therapeutic targets.

Following Victor Zammit's introductory tour around the biochemistry of glucose/fatty acid interactions in the development of insulin resistance, Andreas Kopke (Devgen, Belgium; www.devgen.com) spoke about the utility of an invertebrate model in the search for new drugs modulating this axis. Devgen has capitalized on the extensive literature on *Caenorhabditis elegans* as a model organism for an enormous number of biological processes. These nematode worms are 1 mm, comprised of approximately 1,000 cells and are amenable to liquid handling in microplate format. Molecular pathways are orthologous to 70% of the human pathways represented in the worm genome. Devgen has deployed a variety of knockin/knockout technologies to interrogate effects on a range of phenotypes in high-throughput experiments. Interestingly, these approaches have produced worms with impaired insulin signaling and accumulation of fat droplets, representing a model of obesity that may involve DAF2, the worm equivalent of the insulin-like growth factor-1 receptor. Devgen's proprietary approach in this area is a novel approach to RNA interference technology by genetically engineering the RNAi into *Escherichia coli*, which are eaten by the worms. Subsequently, digestion of the plasmid releases the active RNAi within the worm. A number of druggable targets have been identified by this approach and further validated in mammalian systems. These include two kinase targets with a role in diabetes and obesity. Kinase 1 has subsequently been knocked out in mice and confers protection against high-fat diet-induced glucose intolerance. In addition to biological target validation, Devgen has a library of nearly 100,000 compounds used in lead generation against validated targets. A druggable lead against kinase 1 with an affinity of 400 nM has been identified.

Philippe Froguel (Head, Hammersmith Genome Centre, London, U.K.) delivered the final presentation of the morning. Prof. Froguel reviewed the contribution of genomics and genetics to target identification in type 2 diabetes. One of the driving forces behind this approach is the recognition that current therapies are unable to prevent deterioration of the pancreatic β cell and progression of the disease. Monogenic forms of type 2 diabetes (5 % of all cases) have aided our understanding of molecular determinants of glucose homeostasis. Glucokinase was identified in 1992 by a familial gene linkage analysis. Inactivating mutations in this enzyme, which acts as a glucose sensor in pancreatic β cells and hepatocytes, leads to the development of maturity-onset diabetes. Activators of this enzyme have been identified and show major potential as new drugs for type 2 diabetes. Diabetes, however, is largely a polygenic disease arising from the interplay between genetic and environmental risk factors. Prof. Froquel described some of his work examining whether current diabetes treatments target susceptibility genes. Findings have revealed that variants of the β-cell K_{ATP} channel Kir 6.2 and the sulfonyl urea receptor are associated with diabetes in humans. In contrast, a pro12ala mutation in the PPARγ transcription factor increases insulin sensitivity and is a protective variant. Several single nucleotide polymorphisms in the adiponectin gene have been shown to modulate production and polymerization of this "adipokine," which promotes insulin sensitivity and inhibits hepatic lipid output. Pima Indians in the United States have a high incidence of type 2 diabetes, and it has been reported that the most predictive protective factor in this population is adiponectin levels. Prof. Froquel stressed that a focus on translational research and alliances between academia and industry would be required to make significant breakthroughs in the treatment of type 2 diabetes, leading in the future to rational, individualized prescribing.

Matthew Coghlan (Diabetes and Drug Discovery Team, Astra Zeneca, U.K.) also described glucokinase as a target of interest. Glucokinase is a variant hexokinase, the expression of which is restricted to the pancreas, liver, brain and gut. Glucokinase is physiologically important, as it is the rate-limiting step for glucose uptake into cells. As the kinetics of this enzyme are sigmoidal, its allosteric activity is highly sensitive to glucose concentrations, and insulin secretion is triggered in the β cell at 25% of the V_{max} of glucokinase. Activators of this enzyme, therefore, have the potential to promote glucose uptake and insulin secretion, leading to better control of blood glucose. In addition to evidence from human genetics, this approach has also been validated in transgenic mice carrying an activating mutation of glucokinase. The threshold for glucose-stimulated insulin secretion is reduced in these animals. High-throughput screens subsequently identified **2 [6-[(3-isobutoxy-5-isopropoxybenzoyl)amino]nicotinic acid** and **5-([3-isopropoxy-5-[2-(3-thienyl)-ethoxy]benzoyl]amino)-1,3,4-thiadiazole-2-carboxylic acid** as competitive inhibitors of the closed inactive conformation of glucokinase that increase overall activity. Studies of the mode of action of these compounds indicate that they bind to the enzyme at some distance from the catalytic site and in some respects may reflect changes similar to those in the activating mutation studies in the transgenic mice. *In vivo*, the compounds promote glycogen synthesis in rat hepatocytes and improve the results from glucose tolerance tests. Future studies in humans will determine whether these compounds live up to their therapeutic expectations.

Joel Berger (Merck Research Laboratories, U.S.A.) presented a look into the future of research arising from the discovery of PPAR activation by

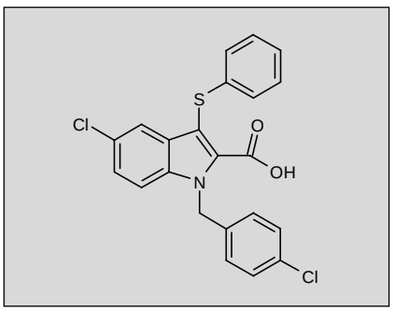

Fig. 1. A unique class of non-thiazolidinedione partial agonists discovered by Merck.

the thiazolidinediones (TZDs) and fibrates. TZDs are agonists of the γ receptor subtype of PPARs, which are ligand-regulated transcription factors. PPARγ is highly expressed in adipocytes. TZDs possess antilipemic and antiglycemic properties and have proved to be efficacious in the treatment of type 2 diabetes for a number of years. However, significant side effects including weight gain and cardiovascular changes have emerged that are restricting more widespread application of these agents. Merck has discovered a unique class of non-TZD partial agonists of PPARγ that have been shown to be less adipogenic than full agonists, according to *in vitro* assays, and to induce a unique gene signature profile (Fig. 1). These compounds are as effective as full agonists in terms of antiglycemic activity in mice, but do not cause cardiac hypertrophy. This class of compound may prove to display a more favorable therapeutic window than full agonists in humans.

A further interesting target highlighted by PPAR research is 11-β hydroxysteroid dehydrogenase-1 (11β-HSD-1). This enzyme is highly expressed in insulin-responsive tissues, where it converts the adrenal hormone corticosterone to the more potent cortisol. Overexpression of 11β-HSD-1 in animals causes insulin resistance, while PPAR agonists inhibit expression of the 11β-HSD-1 gene. The drug discovery team at Merck has taken this observation to the next step and has identified novel inhibitors of 11β-HSD-1 that display antilipemic, antiglycemic and insulin-sensitizing activity in animals. Further, gene microarray studies have led to the discovery of compounds possessing both PPARα and PPARγ activity and favorable effects on the expression of multiple genes known to control lipid metabolism.

Phosphatases as a target for the treatment of diabetes have only recently emerged, although Agnes Bombrun (Serono Pharmaceutical Research Institute, Switzerland) recalled in her talk that clues have been around for many years. **Vanadium**, a phosphatase inhibitor, was first used for the treatment of diabetes in 1899. Protein tyrosine phosphatases (PTPs), in particular PTP-1B, are a well-described component of the negative regulation of insulin receptor signaling and are the focus of a research program at Serono. In mice, disruption of this gene enhances insulin sensitivity and improves glucose tolerance and obesity. In order to be active, phosphatase inhibitors (and several are available for experimental studies) must mimic a dianionic phosphotyrosine. However, once this activity is established the translation from the test tube to *in vivo* treatment raises many hurdles, not least of which is how to deliver such a drug to an intracellular target. The molecular polar surface area versus molecular weight relationships allowed some selection from many of the compounds described in the literature. Using this information, Serono developed novel compounds for further study, although *in vitro* potency was not always carried with drug-like properties such as selectivity or solubility. Using this approach, a number of chemical series were selected (oxindoles, hydrazides, pyrazolidinediones and substituted methylene amides) and carried forward into further testing. Although the structures were not disclosed, several inhibitors were tested in an interesting *in vivo* model of diabetes, the *db/db* mouse, which has a spontaneous mutation in the leptin receptor. These mice are obese, hyperglycemic and hyperinsulinemic, and have impaired insulin sensitivity, which lends itself to being an effective screen for PTP-1B inhibitors. PTP-1B inhibitors with good pharmacokinetics and pharmacodynamics have now been identified and are in the process of more extensive preclinical testing.

Dr. James Foley (Global Clinical Development and Medical Affairs, Novartis, U.S.A.) outlined the company's philosophy toward research in the diabetes area by describing advances in their dipeptidyl-peptidase-4 (DPP-4) program. In the 1990s, Novartis switched focus from trying to stimulate glucose uptake into muscle to focus on dysregulated insulin secretion and impaired suppression of glucagon as key events. Novartis scientists focused on the therapeutic potential of incretin hormones released from the gut and the multiple mechanisms of GLP-1. GLP-1 infusion reduces fasting glucose and improves insulin sensitivity and β-cell function in humans. Animal studies have also revealed a role for the protein GLP-1 in stimulating β-cell neogenesis and decreasing apoptosis. A fundamental problem when looking at GLP-1 as a potential therapeutic is its short half-life (1–2 minutes). Novartis decided to adopt a strategy of searching for inhibitors of the enzyme DPP-4, responsible for degrading GLP-1. The approach was validated when the DPP-4 inhibitor **valine-pyrrolidide** was shown to improve glucose tolerance in rodents and monkeys. Novartis' combichem effort identified the lead molecule in this area, **LAF-237** (Fig. 2), which binds specifically to the catalytic site of DPP-4 and improves the half-life of GLP-1 to about 130 minutes. This compound is a potent (k_d = 5 nM) and reversible inhibitor of DPP-4 that displays good oral bioavailability (~80 %) and does not significantly inhibit P450

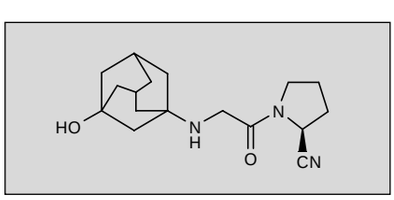

Fig. 2. LAF-237: Inhibitor of dipeptidyl-peptidase 4.

enzymes. Phase II evaluation yielded highly promising results, and LAF-237 is currently in phase III trials. Clinically, LAF-237 shows a durable effect in lowering HbA1c levels in combination with **metformin**. LAF-237 is also effective as a monotherapy and does not promote weight gain. Interestingly, there are other DPP-4 inhibitors of different chemical classes in development by other pharmaceutical companies, which may be a good sign of the utility of these compounds

GLP-1 has also provided the foundation for the discovery of a new promising protein therapeutic being developed by Novo Nordisk, Denmark, its program being presented by Lotte Bjerre Knudsen. GLP-1 has been validated clinically as a potential treatment for type 2 diabetes. Infusions of GLP-1 to patients with type 2 diabetes have been shown to be beneficial in controlling both blood glucose and body weight. Acylation of GLP-1 increases the binding of the peptide to serum albumin and prolongs its bioavailability by protecting the peptide from degradation by DPP-4. Further development of this chemical strategy also demonstrated that extensive acylation as well as modification of the N terminus of the peptide reduced the potency, showing that the opportunities for the discovery of a therapeutically useful compound were limited. **Liraglutide**, Arg(34)Lys(26)-(*N*-ε-(γ-Glu-(*N*-α-hexadecanoyl))-GLP-1(7-37), a peptide with a long-chain fatty acid (palmitoyl) modification was finally chosen as the lead compound with a good pharmacokinetic profile for daily administration. Liraglutide has a bioavailability of 55%, T_{max} of 9–12 hours and half-life of 11–15 hours, and steady state concentrations were reached after three doses. Liraglutide is currently in phase II trials. No serious adverse effects have been recorded, although the dose-limiting factor is nausea. In patients with mild type 2 diabetes, Liraglutide decreases fasting and food-related blood glucose, which was associated with an increased insulin secretion. In obese patients, liraglutide prevented weight gain. Interestingly, in rats liraglutide inhibited food intake, improved food preferences and decreased body weight, data that support the suggestion that these compounds may also play a role in the control of satiety and food intake, a beneficial activity in the treatment of type 2 diabetes.

It is pertinent to close this review of this meeting of the Society for Medicines Research with the observation that there is clearly an unmet need in the pharmacological treatment of type 2 diabetes. The United Kingdom Prospective Diabetes Study shows that after about 2 years of treatment, the current modalities gradually lose effectiveness. We hope that the current research effort so effectively demonstrated by the speakers at this conference will reverse this trend.

The webcast of this meeting can be viewed at: http://webcasts.prous.com/diabetes2004/.

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