ABSTRACT

Diabetic neuropathy is possibly the most frequent complication of diabetes. Even with modern advances, diagnosis and management of neuropathy remains a challenge. Research into the mechanisms that lead to the various complications in diabetes is currently under way in an attempt to identify new targets for treatment, especially in neuropathy. Blood glucose control remains a cornerstone in both prevention and treatment. However, hyperglycemia might not be solely responsible for neuropathy. Four main hypotheses have emerged regarding the pathways leading to endothelial and metabolic dysfunction: increased polyol pathway flux with accumulation of sugar alcohols, accumulation of advanced glycation end-products, oxidative and nitrosative stress, and increased activity of protein kinase C (PKC). Further, the interaction of some or all of these pathways might also present targets for therapy. Insulin and C-peptide deficiencies are viewed as having potential for the development of new treatments, and researchers are also examining the potential for neurotrophic agents to reverse diabetic neuropathy in humans. The research that is furthest along is in the area of PKC-β inhibitors (now in phase III trials), which ultimately may be able to correct several diabetes-induced microvascular complications (eg, retinopathy, nephropathy, nerve damage). The future looks bright that research may provide therapeutic options beyond the current standard of glucose control for the treatment of diabetic neuropathy.

CLASSIFICATION OF DIABETIC NEUROPATHIES

Diabetic neuropathies are complex heterogeneous disorders that encompass a wide range of abnormalities affecting both peripheral and autonomic nervous systems. Diffuse clinical neuropathy falls into 2 distinct groups: distal symmetric sensorimotor neuropathy and autonomic neuropathy. The latter is associated with a long list of dangerous or discomforting effects, including hypotension, enteropathy, cystopathy, and sexual dysfunction, all of which present significant therapeutic challenges.
Focal neuropathies also fall into 2 main subgroups: (1) entrapment syndromes, in which nerves are compressed within small spaces and which are associated with impaired sensation or weakness in the nerve distribution in either the hands or feet, and (2) mononeuropathies, which are caused by vascular insult and resolve spontaneously. The very different course of each condition requires careful distinction.

**Epidemiology**

Two landmark studies published over the past decade—The Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS)—firmly established the connection between blood glucose control and the progression of diabetic microvascular disease. In addition, Dyck et al demonstrated that retinopathy and 24-hour proteinuria are the strongest predictors for diabetic neuropathies, thus solidifying an important link between neuropathy and the other 2 microvascular complications. Dyck et al also demonstrated that fasting plasma glucose, hemoglobin A1c, and duration of diabetes are related to the development and progression of neuropathy. Moreover, the DCCT showed that physiologic glycemic control reduced complications, including neuropathy.

Because this is where the conclusive science has been done, practitioners today center prevention and treatment efforts for diabetic neuropathy almost entirely around blood glucose control. However, recent studies indicate that blood glucose control may prove to be only one piece of preventing and treating neuropathy. It must be remembered that optimum glycemic control reduced the development of neuropathy by 30% to 40%, meaning that 60% to 70% of cases were not accounted for by elevated glucose alone. Indeed, neuropathy may occur in the absence of an elevated fasting blood glucose level.

Three separate studies have looked at patients with painful neuropathy and found that many such patients had impaired glucose tolerance (IGT), a condition that afflicts more than double the number of those who have diabetes and is often a precursor to diabetes. Because the neuropathy associated with IGT is milder than neuropathy associated with diabetes, giving an oral glucose tolerance test at the first sign of neuropathy may enable patients to exert glucose control earlier in the process and thereby better control—or perhaps even reverse—the progression of neuropathy.

Another indication that hyperglycemia alone may not be responsible for neuropathy emerged from a recent study by Sharma et al, where the researchers drew a connection between chronic inflammatory demyelinating polyneuropathy (CIDP) and diabetes mellitus. Researchers found first that patients with diabetes often had a specific type of diabetic neuropathy: CIDP. Moreover, these patients seemed to show significant improvement after treatment with intravenous immunoglobulin (400 mg/kg per day for 5 days). Finally, the researchers looked at patients who presented with CIDP and discovered that they were much more likely to have diabetes than to have other diseases (amyotrophic lateral sclerosis or myasthenia gravis) associated with CIDP. The study may help physicians better understand the exact nature of the neuropathy with which their patients with diabetes present and suggest possible treatments.

In the Rochester Diabetic Neuropathy Study, Dyck et al found that the most significant risk factors for severity of diabetic neuropathy were, in order of importance: diabetic microvascular disease, total hyperglycemic exposure, and type of diabetes.

**Improving Diagnosis**

Herman et al in the Glycemia Optimization with Algorithms and Labs At Point of Care (GOAL A1c) study demonstrated that both endocrinologists and nonendocrinologists failed to recognize mild neuropathy 60% to 70% of the time. There are indications that patients in the early phases of diabetic neuropathy may be more responsive to therapy. However, diagnosis can be challenging, as can be predictions of severity. Recently, there have been advances in this regard.
Sensitive and specific tests do exist for the early diagnosis of diabetic neuropathy and include combinations of vibration perception threshold (VPT) and a thermal test. According to a study by Bril et al, patients with early mild diabetic neuropathy are more likely to have sural nerve action potential that is measurable through the VPT and electrophysiology testing.

**TREATING DIABETIC NEUROPATHY**

Although studies that have narrowed down risk factors for diabetic neuropathy can help physicians to better diagnose and monitor their patients, the therapies currently available to prevent or delay diabetic neuropathy remain inadequate.

The major focus of prevention and treatment has been blood glucose control through diet, exercise, and insulin or oral hypoglycemic agents. Unfortunately, while there is certainly a connection between glucose control and neuropathy, improved glucose control does not seem to prevent or reverse neuropathy in all patients with diabetes. In addition, strict glycemic control is difficult to maintain—hence, the search for new therapies.

Some have noted that blood pressure control, which has had some success in delaying both nephropathy and retinopathy, may have some promise in neuropathy. Malik et al conducted a study using angiotensin-converting enzyme inhibitors and found modest improvements in measures of neuropathic severity but noted that there is a need for large randomized clinical trials before advocating for changes in clinical practice. These have not been performed at this point in time. Furthermore, Gaede and colleagues showed that a multifactorial approach was necessary for mitigating the complications of diabetes.

Most recently, attempts to use aldose reductase inhibitors, antioxidants, advanced glycation end-product (AGE) inhibitors, and PKC-β inhibitors have emerged from our growing understanding of the pathways of microvascular complications. Research in these areas has produced many disappointments and have not yet yielded a proven therapeutic agent. Nevertheless, promise remains, and some new studies seem to justify continuing interest in examining these pathways.

**PATHWAYS AS THERAPEUTIC TARGETS**

Many of the studies that have examined the pathways of hyperglycemia confirmed that hyperglycemia leads to endothelial and metabolic dysfunction, especially increases in vascular permeability, thickening of base membranes, pericyte cell death, and abnormalities in blood flow. The next step has been to understand why those changes occur. Four main hypotheses (discussed in depth by Dr Malik, page S409) have emerged stating that hyperglycemia leads to:

- Increased polyol pathway flux—an increase in the aldose reductase enzyme
- Increased AGE formation
- Increased oxidative stress
- Activation of PKC isoforms

**POLYOL PATHWAY THEORY**

The polyol pathway theory suggests that when glucose concentrations rise to hyperglycemic levels, there is an increased flux through the aldose reductase pathway that enhances the accumulation of sugar alcohols (e.g., sorbitol) that contributes to complications. More specifically, it is theorized that the pseudohypoxia created by increased polyol synthesis reduces the bioavailability of nitric oxide, which leads to decreased nerve blood flow and, in turn, to nerve ischemia and nerve dysfunction. Aldose reductase inhibitors (ARIs) have been effective at preventing some aspects of neuropathy in rats and have even demonstrated improvements in symptoms, signs, and nerve conduction in human clinical trials. Pfeifer et al speculated that ARIs may still prove useful at slowing the progression of distal and autonomic neuropathy and have suggested larger clinical trials. More recently, Obrosova et al found that ARIs prevented retinal oxidative stress and overexpression of vascular endothelial growth factor in rats. Because retinopathy is a predictor of neuropathy, this is an intriguing finding for those treating patients with diabetic neuropathies.

The human trials with ARIs in the past have mostly been disappointing, causing the interest in them as a possible therapeutic option to flag. However, recent trials with epalrestat and fidarestat have rekindled interest in these agents.

**ADVANCED GLYCATION END-PRODUCT THEORY**

The AGE theory began as a way to explain diabetic complications as a form of accelerated aging driven by covalent modification and cross-linking of proteins by glucose. It postulates that formation of AGE may damage cells by impairing the function of a wide range of proteins. It may also alter cellular function by bind-
ing to receptors that can create a series of cellular signaling events that lead to cellular dysfunction.4

Driven by this theory, researchers have tested aminoguanidine, an AGE inhibitor, which was able to block the development of microvascular complications in animals. Clinical trials in human patients have been inconclusive, largely because of the toxicity of aminoguanidine.4

**THE OXIDATIVE STRESS THEORY**

The oxidative stress theory postulates that the metabolism of glucose ultimately leads to accumulation of reactive oxygen species, reduced nitric oxide bioavailability, and damage to cellular proteins and promotes leukocyte adhesion to the endothelium while inhibiting its barrier function. Trials with the antioxidant alpha-lipoic acid have demonstrated improvement in symptoms and objective evidence of neuropathy.25

Bursell et al looked at the use of vitamin E supplements in patients with type 1 diabetes to help control nephropathy and retinopathy, largely because of the hope it would normalize renal blood flow and creatinine clearance.26 The investigators found that vitamin E did help and, in the process, also found a significant association between hemoglobin A1c and good glycemic control. However, vitamin E may neutralize the beneficial effects of statins on macrovascular complications of diabetes,27 and caution must be exercised in prescribing vitamin E in patients with diabetes.

**THE PROTEIN KINASE C THEORY**

Sheetz et al pioneered the notion that hyperglycemia and latterly elevated fatty acids in the absence of hyperglycemia increase the bioavailability of diacylglycerol (DAG), which catalyzes the formation of PKC, an intracellular signaling molecule that regulates many vascular functions.4 The elevated glucose levels associated with diabetes increase glycolytic pathway flux, which ultimately stimulates synthesis of DAG, which in turn activates the PKC isoforms (of which there are at least 12). This theory proposes that activation of PKC in the blood vessels can drive increased permeability, nitric oxide dysregulation, increased leukocyte adhesion, and alterations in blood flow. It can also affect other signaling pathways.

PKC-β, one of the PKC isoforms, seems to be most consistently associated with the main avenues of diabetic microvascular complications. In response, studies have begun to test if PKC-β inhibitors could slow, reverse, or prevent the damage. In the laboratory, a PKC-β inhibitor was shown to block a number of vascular abnormalities, and in rat studies, it seemed to prevent or reverse certain hemodynamic changes associated with microvascular complications, including neuropathy.4 Thus far, human clinical trials seem promising and will be discussed later.

**OTHER THEORIES**

More recently, scientists and physicians have begun to explore 2 new ideas as therapeutic targets. The first postulates that because all of the pathways noted above interact, there may be a way to address all or some of them at once. Brownlee et al noted that all of the different pathways associated with hyperglycemia are connected to a single process, which he calls “overproduction of superoxide by the mitochondrial electron-transport chain.”9 Rather than look at conventional antioxidants, which have been disappointing, many have suggested it might be worthwhile to study small-molecular-weight compounds, including the lipid-soluble thiamin benfotiamine, that could inhibit this overproduction, thus blocking the activation of aldose reductase, AGEs, and PKC and preventing the development and progression of microvascular complications.28

**POLY(ADP-RIbose) POLYMERASE**

An outgrowth of this thinking is seen in work by Du et al and Hammes et al.29,30 In following up on Brownlee’s theory, they have found that superoxide overproduction activates poly(ADP-ribose) polymerase (PARP), which then inhibits glyceraldehyde-3-phosphate dehydrogenase activity but, more importantly, damages DNA leading to the transcription of undesirable mRNA and hence, proteins. By blocking this PARP activity with the use of benfotiamine, researchers found that they effectively blocked hyperglycemia-induced activation of the pathways associated with vascular damage.29,30 This seems to be a promising avenue for further research.

**INSULIN AND C-PEPTIDE**

The second recent theory speculates that in addition to hyperglycemia, insulin and C-peptide deficiencies play a role in diabetic neuropathy, particularly in type 1 diabetic neuropathy. Sima has suggested that impaired nerve regeneration, for example, appears to be mainly the result of impaired insulin action as opposed to hyperglycemia and notes that in patients with type 1 diabetes, autonomic nerve function
Improved after administration of C-peptide. This, too, may offer some promise for further study.

**NEUROTROPHIC AGENTS**

Researchers discovered nerve growth factor (NGF) in the 1950s and, in subsequent years, were able to use it to successfully treat animal models of diabetic neuropathy. In recent years, the biotechnology and pharmaceutical industries could not replicate that success in human clinical trials. Apfel notes that one probable cause of these failures was that agents were administered systemically rather than in a targeted way that more closely replicates the way these factors function naturally.

In another article, Apfel notes that recent attempts to use recombinant human NGF was effective in phase II clinical trials for diabetic polyneuropathy but that painful side effects prevented masking, thereby invalidating the phase III clinical study, which could not confirm the success of the phase II trials. Apfel believes that new techniques that use viral vectors show promise in introducing neurotrophic agents to their target sites, but effectively using these vectors will take time if it is to be workable at all in humans.

Other researchers have looked at the potential for vascular endothelial growth factor (VEGF) to reverse diabetic neuropathy in humans. After encouraging results in the laboratory, Pleasure et al reported that in an open-label trial, patients with critical limb ischemia who were treated with naked VEGF DNA inserted into muscle experienced significant improvement in neuropathic symptoms and sensory perception.

Many questions remain, however, about VEGF. Most important, VEGF has been associated with proliferative retinopathy and can cause peripheral edema in the lower extremity. Still, Vvees et al noted that VEGF is promising because it has the potential to target multiple mechanisms—i.e., it may be able to restore blood flow and promote the survival of peripheral nerve cells. In addition, the simplicity of VEGF gene transfer makes it an appealing candidate for clinical use. There is reason for further studies in this area.

**PKC-β STUDIES**

Although PARP, insulin, and C-peptide all may lead to additional treatments for diabetic neuropathies, it is PKC-β inhibitors that offer the most immediate hope for therapy. Now in 5 phase III clinical trials, PKC-β inhibitors have continued to show promise.

Interest in PKC-β heightened when rat studies demonstrated an ability to correct various diabetes-induced microvascular complications, including nerve damage. Cotter et al used streptozotocin (STZ) to induce diabetes in rats. After 8 weeks, the researchers measured sciatic nerve and superior cervical ganglion blood flow, as well as nerve conduction velocity, finding that diabetes reduced sciatic nerve and superior cervical ganglion blood flow by 50% and produced deficits in saphenous nerve sensory conduction velocity. After 2 weeks of treatment with PKC-β inhibitor ruboxistaurin mesylate (LY333531), the sciatic nerve and ganglion blood flow were almost completely corrected; in addition, the treatments corrected thermal hyperalgesia.

Kim et al also found that PKC-β inhibitor ruboxistaurin improved hyperalgesia on STZ-induced diabetic rats. The researchers found a decreased nociceptive threshold improved after either 4 weeks of treatment with LY333531 or through a single intradermal injection into the footpads. After 6 weeks, they found that the inhibitor improved the decrease in cyclic guanosine monophosphate content in the rats’ dorsal root ganglia and would therefore be effective in treating diabetic hyperalgesia.

Other rat studies looked more specifically at retinopathy and nephropathy. One found that activation of PKC may underlie microvascular complications. Another found that treatment with ruboxistaurin mesylate generated improvements in various features associated with nephropathy.

**HUMAN CLINICAL TRIALS WITH PKC-β INHIBITORS**

Phase 1 and phase II clinical trials with PKC-β inhibitors followed for diabetic retinopathy, diabetic macular edema, and diabetic peripheral neuropathy. Litchy et al found that in a 1-year, double-blind, placebo-controlled, parallel trial, ruboxistaurin mesylate...
improved diabetic peripheral neuropathy. The improvements were evaluated through neurologic examination, objective measures of nerve function, and physician assessment.

In the study, 205 type 1 or 2 diabetes patients with diabetic peripheral neuropathy received either 32 mg or 64 mg of ruboxistaurin mesylate or placebo.

Treatment with the 32-mg dose of ruboxistaurin mesylate produced overall improvement in neurologic examination, particularly in the lower limbs, which are the sites most affected by diabetic peripheral neuropathy, and in the reflexes. Similar improvements in 2 composite scores, which include components of the neurologic examination and electrophysiologic and quantitative sensory tests, were seen in the 32-mg group. Global assessments of patient improvement corroborated these findings.

There were no significant differences in clinical improvement between patients receiving 64 mg ruboxistaurin and placebo. However, people receiving the lower dose had milder neuropathy of shorter duration than patients receiving the higher dose. When subsets of the entire population who had milder neuropathy were evaluated, both doses were effective. Diarrhea was the most common side effect of ruboxistaurin, but in general the drug was well tolerated. There were no effects on glycemic control or hepatic, renal, or bone marrow function.

In an abstract that presented other data from the same study, Vinik et al also reported that ruboxistaurin mesylate improved the symptoms of diabetic peripheral neuropathy and vibratory sensation. The Neuropathy Total Symptom Score-6 (NTSS-6) questionnaire was used to measure symptom improvement in 83 patients. The NTSS-6 measures the frequency and intensity of 6 diabetic peripheral neuropathy symptoms: numbness, allodynia, pricking, and 3 types of pain (aching, burning, and lancinating).

Patients taking both ruboxistaurin doses (32 mg and 64 mg) had improvements in NTSS-6 scores compared with those taking placebo. In a subset of 49 patients with early diabetic peripheral neuropathy and symptoms, a significant change in vibration detection threshold was noted in both groups. The change in this subset correlated with improvement in the NTSS-6.

Finally, Aiello presented phase II and phase III data about retinopathy at the 63rd Scientific Sessions of the American Diabetes Association. While the results for treating retinopathy were inconclusive, the studies did establish that ruboxistaurin mesylate was safe and well tolerated. Five phase III clinical trials are now further studying the efficacy of ruboxistaurin.

**CONCLUSION**

Having made enormous progress in the control of retinopathy and substantial progress with nephropathy, finding effective treatments for neuropathy seems to be the next pressing need in the fight against diabetic microvascular complications. To date, research around PKC-β inhibitors has been encouraging. Should the ongoing phase III trials prove the efficacy of PKC-β inhibitors, we may soon have a legitimate therapeutic option beyond glucose control for the treatment of diabetic microvascular complications.

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