ABSTRACT

For many years clinicians thought that damage to the microvasculature is the underlying cause of diabetic peripheral neuropathy, diabetic nephropathy, and diabetic retinopathy. Now studies have provided considerable epidemiologic, pathologic, pathophysiologic, and molecular evidence to support this long-held clinical hypothesis.

Molecular studies have elucidated the sequence of events in microvascular damage and identified several key pathways (e.g., protein kinase C [PKC], advanced glycation end products, polyol, and hexosamine) that lead to endothelial cell dysfunction and death and, ultimately, to tissue damage in the retinas, kidneys, and nerves. These pathways, therefore, are potential therapeutic targets.

Structural abnormalities in resistance vessels are preceded by functional abnormalities, which are driven by oxidative stress. Superoxide levels are increased and maximal vasodilation is decreased in the streptozotocin diabetic rat. In addition, there is a significant shift in vascular endothelial sensitivity in patients with type 2 diabetes mellitus, and the shift is even more pronounced in those patients with diabetes and hypertension. Impaired vasodilation and an abnormal myogenic response provide no protection to downstream vessels, thus setting the stage for basement membrane thickening and microvascular sclerosis.

There is evidence that basement membrane thickening, a hallmark of diabetic microangiopathy, is present in late-stage diabetic peripheral neuropathy. However, recent studies have shown that it is also present in patients with diabetic peripheral impaired glucose tolerance and early stage diabetic peripheral neuropathy.

Angiotensin II receptor blockade for the treatment of vascular dysfunction partially restores the blunted vasodilatory response and normalizes the shift in blood vessel sensitivity in patients with type 2 diabetes mellitus. Similarly, angiotensin-converting enzyme inhibition results in significant and meaningful electrophysiologic improvements in nerve conduction velocity, M-wave amplitude, and F-wave latency in patients with mild diabetic peripheral neuropathy.

Encouraging data from recently completed studies of the PKC-β inhibitor ruboxistaurin show that it improves symptom scores and nerve function scores in patients with diabetic peripheral neuropathy. (Adv Stud Med. 2005;5(3A):S144-S149)

Diabetic peripheral neuropathy is a degeneration of the peripheral nerves that starts in the distal extremities and then moves proximally. Precisely what causes this type of nerve damage is not yet fully understood, but identifying the cause is the key to developing effective treatment.

Clinicians have thought that damage to the microvasculature could be the underlying cause of not only diabetic retinopathy and diabetic nephropathy but also diabetic peripheral neuropathy. In 1974, Klaes Lundbaek, a practicing diabetologist, proposed the “unitary hypothesis of diabetic microangiopathy” after observing that patients with retinopathy also had some
degree of nephropathy and neuropathy. Without the benefit of molecular studies, he hypothesized that microvascular disease was common to these 3 diabetic microvascular complications.

As described later in this article, there is now considerable epidemiologic, pathologic, pathophysiologic, and molecular evidence to support this clinical hypothesis.

**Epidemiology and Risk Factors**

The Rochester Diabetic Neuropathy Study, a highly acclaimed prospective study of diabetic neuropathy, was conducted by neurologists who examined a broad range of risk factors in patients with different stages of diabetic neuropathy to determine which risk factors had the most impact on outcomes. Assessed risk factors included fasting plasma glucose, glycosylated hemoglobin (HbA1c), and duration of diabetes mellitus—all of which were considered extremely important in the development and progression of diabetic neuropathy.

However, as the investigators surprisingly discovered, the risk factors that most strongly predicted the development and progression of neuropathy were the severity of retinopathy and 24-hour proteinuria. This finding provides strong epidemiologic evidence to support the unitary hypothesis of diabetic microangiopathy.

**Molecular Basis of Microvascular Damage**

Research on the molecular basis of microvascular damage suggests the following sequence of events: excess glucose induces the formation of superoxides that produce mitochondrial oxidative stress. This stress drives several key pathways that are operative in the endothelium (the microvasculature) where they lead to endothelial cell dysfunction and death and, ultimately, to tissue damage in the retinas, kidneys, and nerves. These pathways include protein kinase C (PKC), advanced glycation end products (AGEs), NfκB, polyol flux, and hexosamine flux. Most of the research over the past 25 years has focused on the polyol and AGE pathways, but recent attention has shifted to the PKC pathway.

All of these pathways have a common source (ie, oxidative stress) and a common site where their deleterious effects are exerted (ie, the endothelium). Therefore, blocking these pathways to deter damage to the endothelium is a viable approach to developing an effective therapy for diabetic microvascular complications.

**Microvascular Damage to Peripheral Nerves**

Damage to the endoneurial capillaries supplying the peripheral nerves leads to damage of the nerve fibers and eventually to signs and symptoms of painful diabetic neuropathy or loss of sensation and foot ulceration. Proximal epineurial vessels mediate much of the downstream damage to the endoneurial vessels. Therefore, the crux of the problem is to determine what happens in the epineurial vessels (ie, the arterioles and venules) of the peripheral nerves to permit the progression to diabetic neuropathy.

Because the retina is easily visualized, the vascular abnormalities, such as attenuation of the arterioles, arteriovenous shunting, and new vessel formation, seen in patients with diabetic retinopathy are considered the hallmarks of diabetic microangiopathy. Importantly, and in line with the unitary hypothesis of diabetic microangiopathy, these same epineurial abnormalities have been demonstrated in peripheral nerves.

**Pathophysiologic Factors**

Structural abnormalities in resistance vessels, such as the epineurial arterioles, are preceded by functional abnormalities, which are driven by oxidative stress.

Animal studies have shown that superoxide levels are increased and maximal vasodilation is decreased in the epineurial arterioles of diabetic animals, as compared with control models. However, the same studies also showed that treatment with antioxidants, such as α-lipoic acid, partially restores vascular function in terms of the capacity for vasodilation and the sensitivity of the blood vessel.

Similar research demonstrating functional vascular abnormalities involved human volunteers. However, because epineurial vessels are difficult to obtain from humans, resistance vessels that behave exactly as epineurial vessels have been used instead. In a study by
Schofield et al, vasodilation was clearly compromised in patients with type 2 diabetes mellitus compared to healthy control subjects because of an almost complete absence of the BkCa channel that is key to the mechanism leading to vasodilation. Oxidative stress is the underlying cause of the loss of this channel in the epineurial vessels of patients with diabetes.

When the investigators compared the vascular response to the agonist acetylcholine, they found a significant reduction in maximal vasodilatory capacity in patients with type 2 diabetes mellitus, as compared with that found in the healthy control subjects. Researchers also found a significant shift in vascular endothelial sensitivity in the patients with diabetes. The shift was accentuated further by the presence of hypertension, suggesting that whereas diabetes alone limits the vessel’s capacity to dilate (thus leading to ischemia), diabetes plus hypertension limits the capacity for vasodilation even further.

The shift in sensitivity is, in fact, a double-edged sword. The blood vessels serve 2 purposes: provide blood flow to the tissues and, at the level of the resistance vessels, provide a means of protecting the distal capillaries from too much blood flow (a physiologic attribute of all resistance vessels known as the myogenic response).

As shown in Figure 1, a slow increase in blood pressure flow (passive pressure) increases the luminal diameter of the blood vessel and causes it to dilate in healthy and diabetic individuals. However, when blood pressure outflow is increased quickly (active pressure), the natural response of the resistance vessels is to contract, slow down blood flow downstream, and prevent damage to the distal capillaries. What actually occurs in healthy individuals is that the blood vessel closes down when the blood pressure level is increased quickly, and then the blood vessel re-opens when the blood pressure level is high. By comparison, the vessel continues to dilate in patients with diabetes, providing no protection downstream and setting the stage for basement membrane thickening and microvascular sclerosis.

These changes in vasodilation and myogenic response in diabetic patients, as compared with healthy control subjects, are shown schematically in Figure 2.

**Pathologic Factors**

Basement membrane thickening is a hallmark of diabetic microangiopathy, occurring in the small vessels of the eyes, kidneys, and nerves in patients with diabetes. Although studies show that basement membrane thickening is present in late-stage neuropathy, many in the medical community want evidence that...
basement membrane thickening and other pathologic changes associated with diabetic neuropathy also occur at an early stage of the disease.

An increasing body of medical literature demonstrates that such early changes are present. In 3 studies, approximately 33% of patients who presented to neurologists with painful symptoms suggestive of neuropathy had impaired glucose tolerance; biopsies of sural nerve tissue from these patients clearly showed damage to the small C fibers.\(^\text{10-12}\)

In another study involving 10 patients with impaired glucose tolerance, sural nerve biopsies revealed no loss of large myelinated nerve fibers, but they did show considerable damage to small nerve fibers.\(^\text{13}\) Furthermore, the endoneurial capillary walls showed pericyte and basement membrane changes, with the changes more pronounced in the patients with symptoms of diabetic peripheral neuropathy than in patients without symptoms.

A recently completed study involving 14 patients with type 1 and type 2 diabetes mellitus and minimal or subclinical diabetic peripheral neuropathy at baseline found that pathologic changes in small nerve fibers and endoneurial vessels were present at the early stage of the disease.\(^\text{14}\) Patients in the study had normal vibration sensation, normal thermal discrimination, and near-normal nerve conduction velocity at baseline examination. Sural nerve biopsies were done at this time, and patients were observed for 10 years for progression of neuropathy.

Over the course of the follow-up care, patients showed the typical signs of progression of diabetic peripheral neuropathy; the greatest abnormalities were seen in the unmyelinated small nerve fibers, a finding that is consistent with the early neuropathic changes seen in the small nerve fibers of patients with impaired glucose tolerance.\(^\text{14}\) The biopsies performed at baseline examination also showed that large myelinated fibers, which are the nerve fibers tested in electromyographic studies, were entirely within the reference range for size and number.

Relevant to the argument that microvascular disease is present at the early stage of diabetic peripheral neuropathy, the investigators examined the endoneurial vessels and reported the following results: a 4-fold increase in basement membrane thickness, a 2-fold increase in endothelial cell profile number, and a 50% reduction in the luminal area in the endoneurial vessels in these patients with diabetes and minimal or subclinical neuropathy.

Clinicians surmise that microangiopathy and diabetic peripheral neuropathy are present at an early stage of diabetes mellitus and that microangiopathy progresses with the severity of neuropathy. In other words, microvascular disease becomes more prominent as nerves become more damaged, and vice versa.

The study described earlier in this article demonstrated that late-stage diabetic peripheral neuropathy is the result of changes occurring over several years, and basement membrane thickness and the endothelial cell profile number correlate with the severity of neuropathy in patients with different stages of neuropathy.\(^\text{14}\) These findings support previous research demonstrating that microvascular disease progressively causes basement membrane thickening, a reduction in luminal area, and alterations in pericytes that lead to nerve damage.\(^\text{15}\)

The end result of these changes in peripheral nerves and endoneurial blood vessels are shown schematically in Figure 3. Basement membrane thickening, which occurs in the blood vessel and affects the myelinated and unmyelinated nerve fibers, leads to reduced perfusion and hypoxia in the nerve tissue and, ultimately, to nerve death.
TREATMENT OF VASCULAR DYSFUNCTION AND NEUROPATHY

Because resistance vessels are grossly abnormal in patients with type 2 diabetes mellitus, our research group investigated the commonly used angiotensin II receptor blocker candesartan on the premise that this intervention would restore the blunted vasodilatory response and normalize the shift in blood vessel sensitivity seen in these patients. Patients who had type 2 diabetes mellitus for at least 15 years and normotensive control subjects were randomly assigned to receive candesartan 16 mg or a placebo for 12 weeks; after 12 weeks the patient’s sensitivity was normalized and approximately 50% of the vasodilatory response was restored in the patients with diabetes compared to the control subjects. Importantly, the intervention was able to reverse established vascular alterations in the patients with diabetes.

A few studies have yielded positive findings in the treatment of neuropathy; only 1 study demonstrates benefits with the angiotensin-converting-enzyme (ACE) inhibitor trandolapril in patients with mild diabetic neuropathy. In that study, treatment with the ACE inhibitor for 12 months resulted in significant and meaningful electromyographic changes, with improvements in nerve conduction velocity, M-wave amplitude, and F-wave latency.

However, a review of the molecular basis of diabetic microvascular complications, discussed earlier in this article, suggests several therapeutic targets such as PKC inhibition. Data from early phase II studies of the PKC-β inhibitor ruboxistaurin have been encouraging, as are findings from recently completed and preliminarily reported trials involving patients with diabetic peripheral neuropathy and preliminary trial data evaluating the drug in patients with diabetic retinopathy, diabetic macular edema, and diabetic peripheral neuropathy. Ruboxistaurin had a beneficial effect on symptom scores versus a placebo taken over a 12-month period by patients with diabetic peripheral neuropathy (Figure 4). Also, as compared with a placebo taken over the same time period, ruboxistaurin markedly improved scores of 3 measures of nerve function: nerve impairment scores for the lower limbs, reflexes, and lower limbs plus 4 (Figure 5). The positive effects on both symptoms and nerve impairment are particularly encouraging because they are more likely to promote better adherence to therapy over the long term.

CONCLUSIONS

The key to developing effective treatment for diabetic peripheral neuropathy is to identify the disease’s precise cause. Considerable epidemiologic, pathologic, pathophysiologic, and molecular evi-
dence now support the clinical hypothesis that damage to the microvasculature is the underlying cause of diabetic peripheral neuropathy, diabetic nephropathy, and diabetic retinopathy.

Molecular studies have shown that mitochondrial oxidative stress drives several key pathways involved in the development of diabetic microvascular complications. Therefore, blocking these pathways is a viable approach to therapy. Oxidative stress also drives the functional abnormalities (eg, impaired vasodilation and impaired vascular endothelial sensitivity) that precede structural abnormalities, including basement membrane thickening, in resistance vessels.

Basement membrane thickening, a hallmark of diabetic microangiopathy, is present in early stage and late-stage diabetic peripheral neuropathy and in patients with impaired glucose tolerance. It is more pronounced in those patients with symptoms of diabetic peripheral neuropathy compared to those patients without symptoms. Pathologic changes in small nerve fibers and epineurial and endoneurial vessels are also present in patients with type 1 and type 2 diabetes mellitus and minimal or subclinical neuropathy.

Angiotensin II receptor blockade partially restores the blunted vasodilatory response and normalizes the shift in blood vessel sensitivity in patients with type 2 diabetes mellitus and grossly abnormal resistance vessels. ACE inhibition provides some improvement in nerve function in patients with mild diabetic neuropathy.

The most promising therapy for neuropathy may be the inhibition of the PKC pathway with the PKC-β inhibitor ruboxistaurin. Recently completed studies have shown that this agent improves symptom scores and nerve function scores when compared with a placebo. Additional trials evaluating ruboxistaurin in patients with diabetic peripheral neuropathy, diabetic retinopathy, and diabetic macular edema are currently under way.

REFERENCES