

Clinical Diabetic Neuropathy

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This is the first in a series of articles on presentations at the American Diabetes Association Annual Meeting, San Diego, California, 10–14 June 2005.

A number of presentations at the American Diabetes Association (ADA) Annual Meeting discussed aspects of diabetic neuropathy. At a joint European Association for the Study of Diabetes/ADA Symposium, Rury Holman (Oxford, U.K.) reminded the audience that the U.K. Prospective Diabetes Study (UKPDS) included >5,000 individuals with newly diagnosed diabetes, followed from 1977 to 1991. Trial results were reported in 1998, with 30-year follow-up in progress to further understand the natural history of type 2 diabetes. Holman summarized clinical characteristics of diabetic neuropathy in the UKPDS population. Vibration sensory threshold was measured with a biothesiometer, with a cutoff of 25 V; erectile dysfunction (ED) and foot sensation were assessed by history; ankle and knee tendon reflexes, basal heart rate, and lying and standing blood pressure were determined on examination; and the shortest electrocardiographic RR interval, near the 15th heartbeat after standing from resting, and the longest, around the 30th heartbeat, were measured to give information about autonomic neuropathy. A total of 3,867 individuals had been randomized to conventional versus intensive glucose control from the time of diagnosis of type 2 diabetes, with an HbA_{1c} (A1C) difference of 0.9% between the two groups over 9 years. At 12 years, there were modest although significant differences, with absence of ankle tendon reflexes in 37 vs. 35%, absence of knee reflexes in 12 vs. 11%, and a basal heart rate of 74 vs. 70 bpm. There was a trend to lower risk of sensory neuropathy with better glycemic control. Treatment allocation in the

UKPDS blood pressure substudy had no effect on neuropathy.

In an observational analysis of neuropathy progression in 4,867 people, an elevated threshold for biothesiometer perception was present at the time of diagnosis in 11.4% and developed during follow-up in an additional 12.1% of patients, with a cumulative projected prevalence of 37.3% at 12 years. Women were at lower risk, and there was a 7% increase in cumulative risk of loss of biothesiometer perception for each 1% increase in mean A1C during the study. Loss of one or both ankle reflexes showed a relationship to age, a 25% greater risk in females, and a 5–9% greater likelihood per 1% increase in A1C. There was a history of abnormal foot sensation in 4.1% of patients at entry, showing relationships to age and to male sex, with risk 8% greater for each 1% higher A1C at the time of diagnosis; by 12 years, the risk of subsequent development of loss of sensation was 14% greater per 1% higher A1C. ED was experienced by 19.7% of male study participants at baseline, with risk factors including age and alcohol and cigarette use. Of men without ED at baseline, 34% complained of ED at 12 years, with risk 8% greater per 1% higher level of A1C.

A lower-extremity ulcer had developed in 172 of 4,358 people by 12 years, with risk 38% greater per 1% higher A1C during the study and a 4% increase in risk per year of age. Loss of one or both posterior tibial pulses increased the risk 2.5-fold. There was a fourfold greater risk for each doubling of serum creatinine, a 3% greater risk per kilogram body weight gain, and a 1% greater risk per 10-mmHg higher blood pressure. Current or past cigarette use was associated with an 80% greater risk, abnormal biothesiometer perception with a 90% greater risk, and loss of one or both knee tendon reflexes

with a 120% greater risk of ulceration. Lower-extremity amputation, including that of a digit, was required for 29 of the 2,895 study participants for whom full data were available, with each 1% higher A1C associated with a 57% greater risk, each 10-mmHg higher blood pressure with a 30% greater risk, microalbuminuria at entry with a 2.5-fold risk, absent post tibial pulse with a 3-fold risk, creatinine doubling with a 5-fold risk, and retinopathy at study entry with 5-fold risk. By 10 years, 20% of individuals who had had a leg ulcer required an amputation.

A number of presentations at the ADA meeting addressed further aspects of diabetic neuropathy. Strotmeyer et al. (abstract 1048) studied 2,364 people age 70–79 years. Fourteen percent of individuals with diabetes (comprising 20% of the sample) versus 8% of nondiabetic individuals were unable to perceive the 10-g monofilament, the average vibration detection threshold was 51 vs. 41 μ , and peroneal motor nerve conduction velocity and amplitude were lower in people with diabetes. Reduced vibration sensation correlated with a higher tumor necrosis factor- α in both groups, fasting glucose and A1C correlated with poorer nerve function in individuals with diabetes, and age, arterial insufficiency, height, weight, and male sex associated with poorer nerve function in individuals without diabetes. Idzior-Walus et al. (abstract 31) studied 140 individuals with diabetes, finding that 40% had homocysteine >15 μ mol/l, with mean 17 vs. 13%, and the prevalence of neuropathy among people with homocysteine above versus below 15 μ mol/l was 38 vs. 14%. Wessels et al. (abstract 30) analyzed results of a survey of sexual dysfunction among 587 men who participated in the DCCT (Diabetes Control and Complications Trial). Excluding 55 men who were not sexually active, 143 did and 387 did not report ED, 7.5% reported orgasmic dysfunction, and 36% had decreased libido. A1C, peripheral neuropathy, and creatinine clearance were significant predictors of ED, but participation in the intensive treatment arm of the DCCT was not protective.

Several new agents are becoming available for treatment of painful neuropathy. Rosenstock et al. (abstract 539), Freeman et al. (abstract 551), and Stacey

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Abbreviations: ADA, American Diabetes Association; AGE, advanced glycation end product; ALA, α lipoic acid; ARI, aldose reductase inhibitor; CMT, Charcot Marie Tooth; CTGF, connective tissue growth factor; ED, erectile dysfunction; IL, interleukin; MMP, matrix metalloproteinase; MRI, magnetic resonance imaging; NF- κ B, nuclear factor κ B; PARP, poly(ADP)-ribose polymerase; RAGE, receptor for AGEs; STZ, streptozotocin; UKPDS, U.K. Prospective Diabetes Study.

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et al. (abstract 602) analyzed the effect of pregabalin 150, 300, and 600 mg daily in six trials involving 1,346 people with painful diabetic peripheral neuropathy. They found a dose-related improvement in pain-related sleep interference, with 27, 39, and 46% of patients, respectively, experiencing >50% reduction in pain. After 1 day of treatment, patients receiving placebo or the 150-, 300-, and 600-mg dosages had point reductions of 1.49, 2.04, 2.56, and 2.6 in pain, respectively, based on a 10-point scale. Dizziness and somnolence were reported by 21 and 12% of patients, respectively. In a 15-month open-label study with pregabalin, de Cruz et al. (abstract 564) also reported peripheral edema as a side effect.

Stevens et al. (abstract 891) reported that D-L α lipoic acid (ALA) administration to streptozotocin (STZ)-induced diabetic rats subsequently subjected to a skin abrasion wound showed normalization of wound healing to the rate seen in nondiabetic animals, suggesting a role of this agent in prophylactic administration to individuals with diabetes. Lee et al. (abstract 491) reported that 600 mg daily ALA given orally had similar benefit in reducing symptoms of diabetic polyneuropathy to that seen when initially given intravenously for 2 weeks. Hardy et al. (abstract 465) reported that 28 and 36% of individuals with painful diabetic peripheral neuropathy treated with 60 and 120 mg duloxetine daily, respectively, experienced >30% reduction in pain within the 1st week of treatment, although only 10% of patients completed the 12-week course of treatment and reported being completely pain free, with nausea, somnolence, dizziness, constipation, dry mouth, hyperhidrosis, decreased appetite, and asthenia characterized as dose-related adverse events "frequently reported" in treated patients. Pritchett et al. (abstract 510) and Chappell et al. (abstract 508) analyzed data from >1,100 diabetic individuals with painful peripheral neuropathy treated with placebo versus duloxetine in various doses. At doses of 60 mg once and twice daily, pain was reduced, and functional outcomes such as general activity, walking ability, normal work, and sleep were improved over placebo levels. Raskin et al. (abstract 509), however, reporting on a trial of 348 patients, noted nausea in 19% and somnolence in 12% of treated patients, with 12.1% of those assigned to 60 mg twice daily discontinuing versus 2.6% of those receiving placebo.

Insulin sensitizers may have specific benefit in the treatment of diabetic neuropathy. Schoenauer et al. (abstract 505) reported improvement in cardiac autonomic neuropathy measured by spectral power analysis in 159 people with type 2 diabetes treated with 30 mg pioglitazone daily for 12 months. Yamagishi et al. (abstract 885) studied STZ-induced diabetic rats, showing that motor and sensory nerve conduction velocities decreased 30–35% with STZ, with increase to 80–85% of the nondiabetic level with pioglitazone. Protein kinase C activity was decreased by STZ and restored toward normal with pioglitazone.

Stevens et al. (abstract 881) administered the β amino acid and antioxidant taurine to Zucker diabetic fatty rats. They reported normalization of motor and sensory nerve conduction velocity and nerve blood flow, suggesting a potential therapeutic role in humans. Cameron et al. (abstract 893) administered the inhibitor of κ B kinase 2 AS602868 to STZ-induced diabetic rats, showing correction of the decrease in sciatic motor and saphenous sensory nerve conduction velocity, in sciatic nerve blood flow, and in latency for foot withdrawal from a noxious thermal stimulus, suggesting that the nuclear factor κ B (NF- κ B) system plays a role in neuropathy and that inhibitors could play important therapeutic roles.

Sugimoto et al. (abstract 879) chronically administered insulin to nondiabetic rats from age 3 to 10 months, at a level causing mild hypoglycemia (≥ 2.4 mmol/l) and 14% weight gain. The withdrawal threshold to noxious and non-noxious stimuli decreased and sensory and motor nerve conduction velocities increased, suggesting that insulin, which has potent neurotrophic effects, may play a beneficial role in diabetic neuropathy. McNay et al. (abstract 27) administered insulin into the left hippocampus of freely moving catheterized rats and showed evidence of improved performance on a four-choice maze test. In view of evidence that intranasal insulin improves cognitive function in Alzheimer's patients, the authors suggested that insulin may act affect a variety of central nervous system functions. This observation leads to the important concept that there may be a central nervous system component to diabetic neuropathy. Musen et al. (abstract 896) compared 73 type 1 diabetic individuals (age 33 years, diabetes duration 21 years) with 28 age- and education-matched control subjects and found IQ scores of 114 vs. 119,

with 44 vs. 21% having white matter lesions on magnetic resonance imaging scanning and a trend to association of the number of severe hypoglycemic episodes with such magnetic resonance imaging (MRI) abnormalities. Cukierman et al. (abstract 162) performed a meta-analysis showing diabetes to be associated with a 59% increased risk of dementia, with a 20% increase in cognitive decline based on the Mini-mental State Exam and a 74% increase based on the Digit Symbol Span test. McNeely et al. (abstract 1040), however, studied 111 men with neither diabetes nor cognitive impairment, showing higher verbal and visual memory scores to be associated with higher body fat and BMI, without effect of insulin or glucose levels.

Diabetic foot ulcers and infections

At the International Diabetes Federation/ADA symposium for the "year of the diabetic foot," Andrew Boulton (Manchester, U.K.) presented a "global view of the diabetic foot." He pointed out that "We've got a lot of work to do in the diabetic foot," and emphasized the difficulty of patients not able to experience pain. The prevalence of risk factors such as loss of sensation, vascular disease, and trauma is similar in most areas of Europe, but there has been variability in the rate of change in the prevalence of the condition over the past decade, with 78 and 37% decreases in amputations in Sweden and the Netherlands, respectively, where the number of hospitals with diabetic foot clinics has doubled, but no decrease in Germany and a 24% increase in Lithuania. In Ireland, an audit of one hospital showed that the cost of 30 admissions for diabetic foot ulcers resulting in amputation was ~\$1 million. In the U.S., 60% of lower-extremity amputations occur in individuals with diabetes, and a foot ulcer precedes 85%, with evidence that the cost per diabetic foot ulcer in the U.S. is ~\$28,000. In a Trinidad study of 187 major amputations, 57% were due to diabetic foot infection and 24% involved arterial insufficiency as well as diabetes. In South India, the impact of poverty on diabetic foot lesions is apparent; the prevalence of diabetes is 12.6% in poorer vs. 25.5% in less poor individuals, but neuropathy was present in 62 vs. 43%. The economically disadvantaged group also had increased cardiovascular disease risk. In a study in Nigeria, half of the individuals admitted with diabetic foot problems had major amputation. In an Australian population–

based study of 11,247 individuals, of 367 high-risk patients, only 57% had had a foot exam within the past year. In an audit of foot screening in primary care in New Zealand, of 6,398 patient records, 60% had no record of sensory exam and 58% no record of pulse exam. In Brazil, with an overall prevalence of diabetes of 7.6% and the level much higher in urban areas, the "Save the Diabetic Foot" project during 1992–2002 established a set of diabetes foot clinics, currently with 59 such centers, leading to a 77% reduction in major amputation, although with an increase in "minor" amputations. In China, however, despite the huge population, there are only five foot clinics. Boulton emphasized the need to "remove the shoes and socks, [and] look at the feet."

Zulfiqarali Abbas (Dar es Salaam, Tanzania) discussed foot disease in developing countries. He stressed the importance of diabetes in Africa, with foot lesions a major public health problem associated with prolonged hospitalization. The prevalence of neuropathy is high, at 25–32% in Tanzania, 31% in Zambia, and 28–42% in South Africa. Arterial insufficiency is also increasingly common. An important cause of ulcers in diabetic individuals in Africa is rodent bite, with half leading to amputation. In a large diabetes clinic in Tanzania, of 267 ulcers, 21% had progressed to gangrene at presentation and there was 11% overall mortality. Patients of African and Asian ethnicity had similar presentations, although the former were more likely to have a high glucose level, as well as more evidence of type 1 diabetes, while Asians were more likely to have had stroke and coronary artery disease and were heavier.

Frank LoGerfo (Boston, MA) gave the 2005 Roger Pecoraro Lecture, noting that foot problems cause ~15% of people with diabetes to be hospitalized at some point in the course of the disease. He recalled the first distal bypass he performed >30 years ago, a time when these patients were felt to have what he termed the "myth of microvascular occlusion." "People were just grasping for some explanation" for the frequency of ulcers, he stated; ultimately, no evidence of a microcirculatory occlusive lesion in diabetes was discovered. "You can restore perfusion in these feet successfully," he said. Motor neuropathy particularly affects the innervation of the interossei, leading to the "claw foot," creating pressure points that when combined with limited joint mobility further cause sites of ulceration.

Autonomic neuropathy causes arteriovenous shunting of blood, leading to ineffective circulation, with abnormal cutaneous sweat and oil gland function, leading to skin breaks, which act as portals of entry for infection. Sensory neuropathy results in loss of fine touch as a relatively late manifestation. Abnormal neuroeffector mechanisms lead to altered inflammatory response, with the sensory fiber producing neuropeptides including substance P, which normally mediates the axon reflex but becomes decreased early in diabetic neuropathy. Substance P leads to histamine release from mast cells and results in the granulocyte migration important in the wound healing response. Neuropeptide Y stimulates endothelial cell migration in response to ischemia and stimulates angiogenesis, as well as plays an important role in wound healing. These neuropeptides are diminished in the joint of the diabetic foot, possibly playing a role in Charcot foot deformity, suggesting important roles of neuropathy beyond the traditional motor, sensory, and autonomic functions; this is therefore a potentially important area for research into therapeutic approaches. Clinically, wounds and infections are masked in individuals with diabetes.

Although the microcirculation is not occluded, and there is actually slight enlargement of the capillary lumen, a number of abnormalities are present in vascular function, with thickening of the capillary basement membrane particularly occurring in people with the most advanced neuropathy, an albumin leak paralleling the degree of microalbuminuria (although not impairing diffusion of oxygen), and abnormal endothelium-dependent relaxation (a phenomenon also demonstrable in normal individuals given a glucose load). The combination of decreased vasoactive peptides, abnormal endothelial function, abnormal transcapillary exchange of proteins, and autonomic neuropathy results in abnormal function of the microcirculation, suggesting need for a greater-than-normal total circulation. The standard assessment of the circulation with ankle-brachial index does not take into account "the state of biology of the foot," LoGerfo stated, but he characterized ischemia as "the only aspect of compromised biology that we can change." The enhanced atherosclerosis of diabetes particularly affects the arteries between the knee and ankle, sparing the arteries of the foot, allowing reconstruction to be performed with bypass to the

dorsalis pedis, which "has made a huge difference," with the procedure time intensive but not highly invasive, as it is mainly performed in the subcutaneous tissue. The compromised biology in these individuals, he stated, "argues in favor [of bypass] rather than arguing against it." The use of autogenous veins has been an important component of this treatment. He suggested arteriography in the patient with foot ulcer whenever "you can't feel a posterior tibial or dorsalis pedis pulse."

In a symposium on patterns of diabetic foot infection, Benjamin Lipsky (Seattle, WA) discussed new guidelines (1), suggesting that diabetic foot problems are accorded "lack of respect" by the medical community. He reviewed the need to determine whether a lesion is infected and, if so, how severely; whether wound culture is required and, if so, how to go about doing this; what are the likely pathogens; how osteomyelitis is diagnosed; whether antibiotics are required and for what duration of time; and what is the expected outcome. The International Consensus Working Group on the Diabetic Foot and the Infectious Disease Society of America have described a number of approaches to the problem (see <http://www.iwgdf.org/consensus/wgroup.htm>). Lipsky reviewed a prospective study in the U.K., Sweden, and Germany, showing the tremendous variability in treatment approaches between different regions despite the similarity between patients, suggesting the need for evidence-based guidelines.

Although, Lipsky stated, neuropathy is the major cause of diabetic foot lesions, infection is "the final common denominator that leads most people to amputation." Vascular surgical approaches do appear to have improved, but there is some evidence that the incidence of amputation has actually increased over the past 2 decades. Of people with diabetes, ~25% ultimately develop a foot ulcer, 40% of which are mild, 30–40% moderate, and 20–30% severe. Lipsky cited a prospective study of 1,600 diabetic individuals followed over 2 years, with 9% developing a foot infection, almost all of which were preceded by a wound, with 56% showing evidence of infection, two-thirds involving soft tissue, and ~20% involving bone, 41% requiring hospitalization. The amputation rate in this population was 6 per 1,000 per year, with infection predisposing in 88%, infection increasing the likelihood of amputation 27-fold, and osteomyelitis increasing the risk 8-fold

over soft tissue infection. Wounds penetrating to bone increased risk.

Lipsky suggested an approach to diabetic foot lesions, assessing the systemic response of fever, chills, and glycemia; the patient's psychological status and social situation, with need for adequate home support if bed rest is required; and foot factors, particularly those related to neuropathy. Additional assessment should include the wound size and depth, the presence of necrosis or gangrene, and evidence of bone infection either by probing the wound or by plain X-ray to exclude the presence of subcutaneous air and of bone deformity. The acronym PEDIS has been used to remind the physician to assess the degree of Perfusion, the wound Extent in size and Depth, the degree of Infection, and the presence of Systemic manifestations (2). The extent of infection can be classified as superficial or deep, as grade 1 (none), 2 (superficial), 3 (extensive erythema/depth), and 4 (systemic response including fever). Recognizing that one can be mistaken by the degree of local and systemic response, these approaches allow one to assess the requirement for treatment in a given situation. Lipsky emphasized the need to examine the foot carefully rather than relying on symptoms, given patients' frequent lack of recognition that they have a foot infection.

Describing the approach required for wound culture, Lipsky cautioned against use of a swab by "roll[ing] it over the wound that has an eschar," pointing out that "the real organisms we want to know about are living under that eschar," so that a tissue specimen obtained with curettage of the base of the wound using a scalpel is preferred. Aspirated liquid in the wound, such as purulent material, also gives useful information on culture. With such approaches, even obtained with patients receiving antibiotics, superficial swabs versus tissue show identical results in two-thirds of cases, but approximately one-quarter of swab cultures show bacteria not present in the cultured tissue specimen and one-fifth of swabs not showing an organism that was present in the tissue specimen. Reported culture results vary with the patient population studied. Aerobic gram-positive organisms predominate, particularly *Staphylococcus aureus*, although there is tremendous variability with up to six organisms found per wound. In a multicenter study of 473 tissue specimens, there were only aerobic organisms in half, aerobic and anaerobic organisms in ~45%, and anaerobes only

in <5%. Aerobes included *S. aureus*, usually methicillin sensitive, coagulase-negative *Staphylococcus*, and *Streptococcus*, with enterococcus not infrequent but not always pathogenically important. Anaerobes were predominantly peptostreptococci, responsive to most commonly used antibiotics, as well as to simply opening and debriding the wound, so that the need for antibiotics directed against anaerobes may be less than generally thought. In chronic or previously antibiotic-treated infections, gram-negative rods may play a greater role. Cellulitis without an open skin wound is generally caused by streptococci and occasionally by *Staphylococcus*, infected ulcers by either of these bacteria, macerated ulcers by *Pseudomonas*, and chronic nonhealing ulcers by aerobic gram-positive bacteria.

Virtually all wounds are colonized, but not all are infected. Lipsky noted that antibiotics are not required if there are no signs or symptoms of infection. If one is uncertain as to whether there is clinical infection, or with a prolonged wound, or with significant discomfort, discharge, or odor, these agents are required, almost always in conjunction with surgical debridement. A reasonable approach is to begin with a broad-spectrum antibiotic regimen and then give a more specific agent when cultures are ready. No single antibiotic has been generally superior to all others. Local therapy, both topical and with implanted antimicrobial-soaked sponges now being studied, is another important approach, although one must demonstrate to the patient the proper technique for successful use, as well as educate the patient in dressing changes and other self-care modalities. A number of adjunctive treatments are being explored, including the use of maggots, leading to fewer amputations and lessening the duration of antibiotic requirement; hyperbaric oxygen, which was shown to reduce amputation rates in a recent Cochran review (3); and granulocyte colony-stimulating factor, which does not improve the rate of infection resolution but reduces lower-extremity amputation rates.

John Embil (Winnipeg, Canada) discussed controversies in the diagnosis and treatment of osteomyelitis, suggesting that its diagnosis is not straightforward, requiring clinical skills and radiologic evaluation, with remission rather than cure often the goal, with bone debridement needed for extensive disease, for de-

formity, or for failure to respond to medical treatment. In the diabetic foot, osteomyelitis is typically due to direct inoculation of infection, typically *S. aureus*, into bone, occurring in ~15% of diabetic individuals with foot ulcer. Diabetes control, nutrition, and neuropathy are host factors. The plain radiograph is, Embil stated, quite useful in assessing osteomyelitis; nuclear imaging is not sufficiently sensitive, and magnetic resonance imaging is probably a superior approach if available. In a series of 54 infected feet, two-thirds with ulcer, 16% had underlying osteomyelitis. Embil suggested that the concept that probing to bone is sufficient for the diagnosis of osteomyelitis is based on "old data," and may not have sufficient specificity, although a negative test usually does exclude the diagnosis. There are few prospective trials of therapy. In a study of treatment with clindamycin, 78% of specimens remained positive after 14 days vs. 15% after 28 days, suggesting that the longer treatment period is required. Prolonged treatment with oral antibiotics may be effective, although there is some question as to whether this approach leads to a greater frequency of relapse. Often, surgical debridement of areas of nonviable bone or morphologic abnormality is required.

A number of presentations at the meeting addressed additional aspects of diabetic neuropathy and lower-extremity ulcers in people with diabetes. Greenman et al. (abstract 79) performed ³¹P MRI studies, finding that metatarsal head muscle-to-total cross-sectional area ratios were 55% in individuals without diabetes and 44% in those with diabetes without clinical neuropathy; however, they were 6% in those with neuropathy, with the ratio correlating strongly with the neuropathy disability score, vibration perception threshold, and Semmes-Weinstein monofilament neuropathy scores. Muscle atrophy may then be seen early in the course of diabetic neuropathy and may play a greater role than generally appreciated in the development of diabetic foot lesions; the measurement may be useful in assessing the response to new forms of treatment. Showing the importance of regional differences in care patterns, Mountford et al. (abstract 1103) reported that lower-extremity amputation rates in South Carolina, which were 38 per 1,000 diabetes hospitalizations in 1997, as opposed to the rate of 21 per 1,000 nationally, decreased in 2002 to 18 per 1,000, the same rate as that seen nationally. The

authors attributed the improvement to an educational awareness intervention. African-American males appear to be at highest risk, with Jenkins et al. (abstract 198) reporting that in Charleston, South Carolina, the amputation rate in this group decreased from 79 per 1,000 diabetes hospitalizations in 1999 to 32 per 1,000 in 2002 in the context of the health care provider, patient, and community educational intervention.

Wunderlich et al. (abstract 200) reported characteristics of 81 diabetic individuals with venous insufficiency leg ulcers measuring 2.8 cm² at baseline. Mean age was 72 years, diabetes duration 13 years, and BMI 36 kg/m². The use of compressive dressings, changed weekly, in the 74 people with ankle-brachial index >1.0, led to healing on average in 13 weeks. Over 18 months, 63% of ulcers recurred, but the recurrence rate was just 12% among individuals complying with recommended use of medical-grade compression stockings. (Of course, this may be a proxy measure of overall compliance.) Schwegler et al. (abstract 66) assessed 20 diabetic individuals with foot ulcer for >8 weeks, using MRI, ¹⁸F-deoxyglucose positron emission tomography, and ^{99m}Tc-monoclonal antibody antigranulocyte scintigraphy, showing MRI to identify seven patients with osteomyelitis, which was subsequently proven by biopsy. The diagnostic finding was clinically significant because six of the seven patients required either amputation or had chronic (>24-month) ulceration. Only two of the lesions were identified by the other imaging modalities. Lavery et al. (abstract 65) followed 1,666 individuals with diabetes for 27 months, with 247 developing a foot ulcer and 151 developing 186 foot infections (20% involving bone). Peripheral arterial disease increased the likelihood of osteomyelitis 4.6-fold, location of the ulcer on the lesser toes or metatarsals increased osteomyelitis 8.2- and 8.7-fold, respectively, history of previous amputation increased osteomyelitis 6-fold, and a history of recurrent ulcers increased osteomyelitis likelihood 3.3-fold. Inability to probe to bone was associated with 98% likelihood that osteomyelitis was not present, although only 57% of probe-positive ulcers had underlying osteomyelitis.

Experimental diabetic neuropathy

Angelika Bierhaus (Heidelberg, Germany) discussed signaling and diabetic

neuropathy, reviewing the multiple molecular mechanisms of nerve damage, including protein kinase C activation, oxidative stress, and increased glycation and advanced glycation end product (AGE) formation. Hyperglycemia results in superoxide generation, contributing to formation of AGEs, which bind to the receptor for AGEs (RAGE), causing cellular signaling. In sular nerve biopsy, hyperglycemia and, to a lesser degree, insulin resistance increase AGE, RAGE, interleukin (IL)-6, and NF- κ B. Studying the roles of NF- κ B and inflammation, STZ-induced diabetic rats had NF- κ B-dependent IL-6 production. AGE administration and RAGE activation replicated the effects of hyperglycemia, while RAGE antibody and ALA administration reduced these phenomena. In isolated dorsal root ganglia, carboxymethyllysine and AGE effects were not seen in tissues from mice not expressing RAGE. Diabetes-induced NF- κ B activation also was not seen in the RAGE-null animals. The latency of response to noxious stimuli was increased in diabetes, with RAGE-null mice appearing to have partial preservation of pain perception, although not full restoration, suggesting that other mechanisms exist for development of diabetic neuropathy. However, administration of the circulating form of the receptor, soluble RAGE, did reverse the loss of pain perception, suggesting additional effects beyond reduction in RAGE receptor activation. She concluded that RAGE plays a role in the effect of diabetes on the nervous system via an NF- κ B-related inflammatory process, leading to elaboration of factors such as IL-6. She hypothesized a vascular component, with perineural vessels involved in the process, perhaps with oxidative stress initiating the pathway to RAGE activation.

Klaus-Armin Nave (Max Plank Institute, Goettingen, Germany) discussed aspects of neurologic dysfunction, reviewing the normal function of glial cells in preserving the myelin sheath of the axon, with signaling from the axon leading to myelin production by Schwann cells, a site of potential impairment in diabetic neuropathy. The myelin sheath is similar in the central and peripheral nervous system, although different proteins are involved, so that mutations are selective for disease of one or the other system. Neuropathy may be caused by a number of genetic defects, referred to collectively as Charcot-Marie-Tooth (CMT) diseases, potentially shedding light on diabetic

neuropathy. CMT1 is autosomal or X-linked dominant and involves glial cells, whereas CMT2 involves axonal dysfunction, with different genes affected. The former involves genes that affect myelin and the latter axonal motor proteins. CMT1A maps to chromosome 7 and is caused by a gene duplication, leading to a 1.5-fold overexpression of a glial cell membrane protein, which is retained in the endoplasmic reticulum, leading to axonal thinning and eventual loss. Oxidative stress and nutrient deprivation can lead to similar peripheral nerve cell abnormality. Nave suggested that the Schwann cell in diabetes may have a similarly altered ability to correctly fold membrane proteins. Molecular chaperones in glial cells are involved in protein folding and are highly sensitive to metabolic dysfunction, suggesting roles as mediators of diabetic neuropathy. Nuclear progesterone receptors mediate myelin protein expression, with progesterone administration worsening and progesterone antagonists potentially improving disorders of overexpression, such as the CMT1A animal model. The use of such agents can lead to axonal preservation in this animal model.

At a symposium on animal models of diabetic wound healing, it was noted that the diabetic foot ulcer is a chronic ulcer, differing from the acute wounds that are more often studied in animal models. Furthermore, unlike in human diabetes, animal models with severely uncontrolled diabetes may be in a catabolic state. The adverse effects of neuropathy in wound healing, as well as potential detrimental effects of inappropriate expression of growth factors/cytokines, are additional important factors to be considered in understanding this complex condition. Kelman Cohen (Medical College of Virginia) discussed the need to fully characterize the wound-healing process. Clinical concepts include the separate processes of epithelialization, contraction, and matrix production, all of which play roles in wound healing. Cohen suggested determining the factors that initiate the diabetic wound and those that prevent healing of the chronic wound, as well as the implications of these factors, for approaches to promote wound healing in diabetes. He discussed the effects of pressure, glycation, nitric oxide, and abnormal leukocyte function and cell adhesion. He noted that proteases are present in a high level in chronic human wounds, perhaps destroying cytokines and cytokine receptors, perhaps interfering with

normal healing as well as with clinically available products such as becaplermin gel. Stephen Twigg (Sydney, Australia) discussed a baboon model of wound healing. At 4 years of STZ-induced diabetes, with glucose levels of 15–25 mmol/l, microalbuminuria becomes common, with renal biopsy evidence of increase in glomerular matrix, electron microscopic changes in neurons, less autoregulation of blood pressure with standing, skin biopsy showing abnormal cutaneous vessels, and fewer myelinated nerve fibers. Wound healing is characterized by abnormal blood vessels and increased neutrophils with decreased macrophage infiltration. Connective tissue growth factor (CTGF) induces extracellular matrix, is increased by transforming growth factor- β , and is decreased by inflammatory cytokines. Matrix metalloproteinases (MMPs) are secreted in a proform and activated on the cell surface, with evidence that CTGF inhibits MMP activity, decreasing matrix degradation. Granulation tissue does not increase normally in diabetes, and lack of CTGF as an important trophic factor in the diabetic wound may lead to excess MMP activity. In the diabetic baboon model, CTGF levels are decreased, possibly playing a role in poor wound healing.

Csaba Szabo (Budapest, Hungary) discussed the role of poly(ADP)-ribose polymerase (PARP) in diabetic neuropathy. PARP is a nuclear enzyme present to an extent next only to DNA and histones, playing a role in the maintenance of genomic integrity, normally in a low activity state until activated by DNA breaks. PARP recruits DNA ligase and other reparative enzymes to sites of DNA strand breaks, leading Szabo to caution that PARP inhibitor treatment might be mutagenic with long-term use. PARP cleavage is a marker of apoptosis. PARP1, the major isoform, has zinc fingers recognizing DNA breaks, cleaving NAD and building (poly)ADP-ribose chains, thereby leading to cellular acidification. Excessive PARP activation may, however, lead to cellular necrosis by causing NAD depletion, while PARP inhibition can inhibit this. Administration of PARP inhibitors in animal models restores endothelial function, NADPH pools, and nitric oxide generation.

Diabetes appears to lead to high levels of PARP activity. Endothelial cells incubated in high glucose show increased levels of reactive oxygen species with consequent DNA single-strand breaks and PARP activation (4). In a galactose-treated neuropathy model, either use of

animals not expressing PARP or administration of PG34, a compound that inhibits PARP, prevents the development of neuropathy, suggesting a pathogenic role. PARP activation is present in both Schwann and endothelial cells. Peroxynitrite decomposition catalysts, which prevent PARP activation, are also beneficial in models of neuropathy. There is evidence that combination treatment with PARP inhibitors and ACE inhibitors or β -blockers can reduce neuropathy. Ongoing studies are addressing effects of the agent on ED and a number of other potential clinical applications (5).

Several reports at the meeting addressed aspects of the relationship between PARP and diabetes. Obrosova et al. (abstract 870) presented *in vivo* studies of the PARP inhibitors 3-aminobenzamide and 1,5-isoquinolinediol, showing that both agents reduced the elevation in aortic and epineurial vessel superoxide production in diabetic rats, suggesting that PARP activation is not only caused by oxidative stress but may itself worsen this process. Kaur et al. (abstract 664) demonstrated increased PARP mRNA expression in hearts from STZ-induced diabetic rats, demonstrating *in vitro* that 25 mmol/l glucose upregulated PARP mRNA, a process inhibited by protein kinase B and C and mitogen-activated protein kinase inhibitors, as well as by a specific PARP inhibitor, suggesting that multiple signaling mechanisms can lead to PARP activation in diabetes. In a clinical study, Shrikhande et al. (abstract 686) administered 160 mg valsartan daily to 13 people with type 2 diabetes and 13 control subjects, with forearm skin biopsies showing that valsartan decreased PARP immunoreactivity in 50% of those with diabetes but none of the control subjects, potentially explaining the 60% increase in resting skin blood flow seen only in the diabetic individuals.

Therapeutic agents in the treatment of diabetic neuropathy were studied in a number of further presentations at the meeting. The enzyme aldose reductase may play a role in the development of diabetes complications. Donaghue et al. (abstract 26) studied 216 adolescents with type 1 diabetes, showing that those with the Z+2/Z+2 genotype of aldose reductase had reduction in development of peripheral and autonomic neuropathy. In a study of 262 Japanese individuals with type 2 diabetes, Nakashima et al. (abstract 643) reported that the T allele of the -106C/T promoter region of the aldose

reductase gene, which was accompanied by increased erythrocyte aldose reductase protein, was associated with an increased risk of cerebrovascular disease.

Matsumoto et al. (abstract 475) reported that the aldose reductase inhibitor (ARI) ranirestat (AS-3201) normalized nerve sorbitol levels and improved nerve dysfunction in diabetic rats and in patients with diabetic sensorimotor polyneuropathy. Bril et al. (abstract 488) reported that a 48-week extension study with the agent in humans was associated with improvement in peroneal nerve motor conduction velocity and in sural and median sensory nerve conduction velocity. Hotta et al. (abstract 872) reported a 3-year trial of the ARI epalrestat (150 mg daily) versus placebo in 594 people with diabetic neuropathy. Subjective numbness of upper and lower extremities, paraesthesia and cramping improved, and motor nerve conduction velocity and vibration perception threshold showed stability versus worsening, with particular benefit for people with A1C <7% and for those with no more than mild retinopathy and those without albuminuria, suggesting a role for individuals with good glycaemic control and for those without retinopathy and nephropathy. The ARIs also may have benefit in diabetic nephropathy, with Oates et al. (abstract 797) finding that STZ-induced diabetic rats treated with the ARI zopolrestat or ARI-809 or the sorbitol dehydrogenase inhibitor SDI-931, showing 69, 81, and 60% decrease in albuminuria, with the improvement proportional to the decrease in urinary sorbitol excretion. Obrosova et al. (abstract 809) reported that fidarestat reduced diabetes-associated renal cortex sorbitol and fructose accumulation, and decreased PARP, suggesting an interesting association between the two mechanisms of development of diabetes complications.

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