Diabetic Retinopathy and Neuropathy

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Mechanisms of diabetic retinopathy

Holger Gerhardt (Goteborg, Sweden) discussed pericytes and vascular stability in the diabetic retina, noting that these cells cover most of the endothelium and are present during the development of normal new blood vessels, while proliferative retinopathy classically shows "pericyte drop out" as an early pathological marker. It has been hypothesized that endothelial dysfunction leads to capillary occlusion and regression, causing ischemia, to which neovascularization with subsequent proliferative retinopathy is a response. In this schema, pericyte drop out may occur either before or after the stage of endothelial dysfunction. A number of markers can be used for characterizing pericytes. Mice that do not express platelet-derived growth factor (PDGF)-β have severe diffuse bleeding and die early in the neonatal period. There is diffuse pericyte loss in these mice, suggesting PDGF-β to be an important growth factor for the cells. An endothelium-specific PDGF-β knockout displays a variety of phenotypes with variable degrees of pericyte loss, with areas of pericyte-covered vessels and areas lacking pericytes and with severe retinopathy localized to the areas lacking pericytes. Gerhardt suggested that pericyte drop out is therefore sufficient to cause capillary occlusion and regression, leading to retinal ischemia. Why, though, he asked, is there a proliferative response rather than normal development of well-behaved new vessels repairing the region of ischemia, and how does retinal normal vascular development differ from that seen in neovascularization? The normal advancing vascular sprout extends "tip cells," which appear to guide the development of the vasculature, following paths produced by astrocytes, which produce vascular endothelial growth factor (VEGF)-α in areas of hypoxia, with astrocyte VEGF-α downregulated after it becomes covered by new vessels. In the developing retina, the VEGF receptor (R)2 is present only on the tip cells. If soluble VEGF-R (neutralizing VEGF-R2) is injected in this model, the tip cells regress. In mice that lack the heparin-binding form of VEGF-α, and only produce a diffusible form, VEGF-α is present throughout the retina with loss of patterning orientation of vessels, leading to "misguidance of the tip-cell filopodia," suggesting that heparin-binding isoforms of VEGF play an important role in normal vascular growth. In a neonatal high (75%) oxygen–induced retinopathy model, VEGF is downregulated, leading to vessel regression, thereby producing a form of retinopathy similar to the retinopathy of prematurity developing on subsequent exposure to 25% oxygen. At the onset of neovascularization in this model, the filopodia are lost with consequent diffusion of the endothelial proliferative response to produce retinopathy, with formation of vascular tufts extending into the vitreous rather than penetrating normally into the retina reflecting the loss of directed tip cell filopodia and redistribution of VEGF protein from its site of production. Gerhardt showed that matrix metalloproteinase (MMP) inhibition restored VEGF distribution and guided angiogenesis in this model, suggesting that increased protease activity in the retina was related to the findings. Injection of MMP-9, on the other hand, mimicked the disturbed VEGF-A gradients in the developing retina, suggesting a role of macrophages in producing the abnormal retinal revascularization at the terminal hypothesized step of neovascularization causing proliferative retinopathy. Macrophage MMP production may also play a role in the earlier steps of pericyte drop out.

Gavin Thurston (Tarrytown, NY) discussed the role of angiopoietin (Ang) in diabetic retinopathy. PDGF, VEGF, and the Angs are vascular-specific growth factors. Even in avascular tissues such as the cornea, introduction of VEGF via a viral vector can induce an angiogenic cascade. Strategies that have been developed to block the VEGF pathway include Avastatin, a recombinant humanized antibody to VEGF (recently approved for treatment of colorectal cancer), antibody to VEGF-R2, and a soluble recombinant decoy VEGF receptor, VEGF Trap, which blocks angiogenesis and reduces tumor growth in a rodent model. VEGF appears to initiate angiogenesis via VEGF-R2, creating a primitive vascular plexus, with additional factors/receptors resulting in remodeling to produce more specialized mature vessels. Genetic models not expressing the initial stages of these vascular growth factors ("knockouts") lead to failure of primary vasculature developing, while in the later stage knockouts prevent vascular maturation.

Ang-1, -2, -3, and -4 bind to Tie receptors (so termed because they are *t*yrosine kinases that contain *i*mmunoglobulin and *e*pidermal growth factor domains). Ang-1, -2, and -3 bind to Tie-2 and are essential for vascular development. They are also involved in cardiac and vascular remodeling and in pericyte association with endothelial cells. Transgenic cutaneous overexpression of Ang-1 results in reddened skin with enlargement of its vasculature but with resistance to inflammation-induced increase in vascular permeability, while mice with cutaneous VEGF overexpression, which causes proliferation of small vessels with increased vascular permeability, develop

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Abbreviations: ABI, ankle-brachial index; Akt, protein kinase B; ALA, α-lipoic acid; Ang, angiopoietin; BPI, bactericidal/permeability-increasing protein; CAN, cardiac autonomic neuropathy; CD, cluster of differentiation; JAM, junctional adhesion molecule; Mac-1, macrophage-1 antigen; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; PECAM, platelet endothelial cell adhesion molecule; PKC, protein kinase C; PTA, percutaneous transluminal angioplasty; PVD, peripheral vascular disease; RAGE, receptor for advanced glycation end products; VEGF, vascular endothelial growth factor; VEGF-R, VEGF receptor.

psoriasis-like lesions. Double Ang-1 plus VEGF overexpression suppresses the VEGF-induced leak. Thus, Ang-1 could be seen as an endothelium-specific anti-inflammatory factor. Ang-2 is an agonist at Tie-2 on fibroblasts, is induced both in situations of vascular growth and of vascular regression, and acts as an antagonist of Ang-1 on endothelial cells. Ang-1 is made by perivascular cells, while Ang-2 is made by endothelial cells. Mice with Ang-2 knockout appear to have normal vascular development but develop edema and chylous ascites associated with defects in the lymphatic vasculature. Lack of Ang-2, however, prevents animal models of neovascularization, leading to the concept that in blood vessels, VEGF-α acts at VEGF-R2, with Ang-1 acting at Tie-2 and Ang-2 antagonizing the Ang-1 effect. In lymph vessel formation, VEGF-C acts at VEGF-R3, causing lymph vasculogenesis and sprouting with subsequent maturation induced by Ang-2.

At the cellular level, both Ang-1 and VEGF similarly induce mitogen-activated protein kinase, but Ang-1 more strongly induces protein kinase B (Akt). One of the key downstream mediators of Akt comes from the FOXO (Forkhead) transcription factors, so that Ang-1 may promote survival and stabilization of endothelial cells as well as induce other effects by acting via Akt to phosphorylate Forkhead, reducing its action. One downstream effect of Forkhead is Ang-2 production, suggesting a feeback loop whereby Ang-1 blocks Ang-2.

In STZ-induced diabetic retinopathy, Ang-1 both acutely and chronically normalized retinal ICAM (intercellular adhesion molecule) and VEGF mRNA and protein levels, as well as reducing blood-retinal barrier breakdown, with decrease in adherent leukocytes and in endothelial cell loss. In a number of models, Ang-1 acts to decrease inflammation, although not appearing effective in various disease models with chronicity or with a strong infiltrating cell component. Thurston concluded that VEGF is the key upstream initiator of angiogenesis, while Ang-1 is necessary for vascular development, may play a role in vessel stabilization, can enlarge vessels, and has anti-inflammatory effects, which may decrease diabetic retinopathy. Ang-2 has a role in destabilizing in vessel remodeling and in lymphatic vessel growth.

Triantafyllos Chavakis (Heidelberg, Germany) discussed leukocyte-endothelial interactions in retinopathy. Leukocytes initially tether to and then "roll" along endothelial cells via interaction of selectins, integrins, and other adhesion molecules in both the endothelium and leukocyte. The rolling leukocyte then becomes activated by chemokines, flattens out, and firmly adheres to the endothelial surface in preparation for transmigration through the interendothelial cell junction, a less well understood process. VEGF increases leukocyte adhesion in the diabetic rat retina, with VEGF blockade inhibiting pathologic proliferative retinopathy and also blocking leukocyte adhesion to the endothelium. Cluster of differentiation (CD)18 blockade suppresses integrin action and decreases hyperoxia-induced vaso-obliteration. The receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily of cell surface proteins that has been shown to be a ligand of β_2-integrins mediating leukocyte adherence. RAGE ligands include carboxymethyl lysine, hydroimidazolones, S100 proteins (so termed because of solubility in 100% saturated ammonium sulfate solution), and amyloid. RAGE activation leads to a number of inflammatory vascular complications of diabetes, such as the increased expression of adhesion molecules. RAGE directly mediates adhesion of leukocytes to endothelial cells, with the secreted isoform, soluble RAGE, acting as a decoy to prevent rage signaling in this and other processes. RAGE-dependent adhesion of leukocytes to endothelial cells is mediated by a direct interaction of RAGE with the β_2-integrin macrophage-1 antigen (Mac-1), with RAGE/Mac-1 interaction augmented by S100 protein. In vivo, RAGE knockout mice show decreased neutrophil and monocyte/macrophage recruitment and, in particular, fail to show the increased leukocyte recruitment seen in diabetic models with normal RAGE expression.

Transendothelial leukocyte migration must pass the zonula occludens (tight cell-cell junctions that seal adjacent epithelial cells together), and zonula adherens (anchoring points, or belts in sheets of cells, where the cytoskeleton of neighboring cells are connected to each other). The passage of leukocytes through these junctions without causing endothelial cell leakiness appears to involve interaction with platelet endothelial cell adhesion molecule (PECAM)-1, also termed CD31, an immunoglobulin superfamily molecule found on neurtrophils, monocytes, and platelets that rapidly recycles from the subjunctional reticulum to the endothelial junctions and surrounds transmigrating leukocytes. Blocking PECAM-1 with an antibody inhibits the recycling of PECAM-1 and blocks leukocyte transmigration. CD99 is another molecule involved in transmigration. It is found on most hematopoeitic cells and appears to mediate a terminal step in leukocyte transmigration. Blocking CD99 leads to arrest of leukocytes partway through the endothelial layer. The junctional adhesion molecules (JAMs) are another important family of molecules that also belong to the immunoglobulin superfamily, consisting of two immunoglobulin domains found at tight junctions of endothelial and epithelial cells. JAMs contain a COOH-terminal PDZ domain (a protein-recognition domain binding to the C-terminal end of target proteins), leading to interaction with molecules of the tight junctions. JAM-C localizes at interendothelial contacts and is a counter-receptor of Mac-1, as well as directly binds to Mac-1, and mediates neutrophil transmigration. Thus, understanding of the complex interaction of pericytes, leukocytes, and vascular growth factors appears to offer an understanding of the processes leading to diabetic retinopathy, with, ultimately, the development of therapeutic approaches.

A number of research presentations at the ADA meeting gave further information regarding processes contributing to diabetic retinopathy. Biarnes Costa et al. (abstract 100) demonstrated upregulation of 14 genes coding for acute-phase response proteins in retinal Müller glial cells of streptozotocin-induced diabetic rats, with direct demonstration of 3.0- and 5.1-fold increase in expression of mRNA and 3.9- and 1.7-fold increase in expression of protein of α-2 macroglobulin and ceruloplasmin, respectively, as well as a 10.4-fold increase in interleukin-1β mRNA. Zhang et al. (abstract 102) reported that overexpression of Akt in retinal Muller cells cultured in 25 mmol/l glucose for 72 h reduced apoptosis, suggesting this to be an anti-inflammatory prosurvival pathway. Grant et al. (abstract 96) described studies of the role of SDF-1 (stromal-derived factor-1), showing that in a retinal vessel occlusion model of neo-

vascularization, it increased endothelial cell expression of VCAM-1 (vascular cell adhesion molecule-1) and VEGF, decreased the tight junction protein occludin, and increased migration of endothelial cells and their precursors, with antibody to SDF-1 decreasing neovascularization. Dohoghue et al. (abstract 97), from the same group, reported that the receptors for VEGF and for IGF-1 are both involved in decreasing expression of occludin by high glucose, a component of increased vascular permeability leading to retinopathy. Inhibition of IGF-1 action appeared to be more effective than inhibition of VEGF action in decreasing neonatal hyperoxia-induced neovascularization. Yagamata et al. (abstract 98) reported that bactericidal/permeability-increasing protein (BPI) is expressed in the retina with a role in survival and function of retinal microvascular pericytes and pigment epithelial cells and antiangiogenic actions. Vitreous BPI levels were lower in active diabetic proliferative retinopathy than in vitreous from patients with inactive retinopathy or from patients without diabetes, and intraperitoneal BPI administration decreased neovascularization by 42% in the ischemic neonatal mouse retina model. Zheng et al. (abstract 99) reported increased activity of the nuclear DNA repair enzyme PARP [poly-(ADP-ribose) polymerase-1] in whole retina, endothelial cells, and pericytes of diabetic rats. Addition of the PARP inhibitor PJ34 to retinal endothelial cells incubated in 25 mmol/l glucose reduced nuclear factor κB binding to DNA, suggesting an anti-inflammatory effect, as well as reducing cell death, and administration of PJ34 for 9 months to diabetic rats reduced retinal microvascular cell death and development of early lesions containing acellular capillaries and pericyte ghosts. Lin et al. (abstract 103) reported that after 30 weeks of diabetes the antioxidant dexlipotam, R^+ α-lipoic acid (ALA) reduced acellular-occluded capillaries 2.9-fold and pericyte loss 1.5-fold, suggesting this to be another mechanism of retinopathy. Inoue et al. (abstract 887) showed that prolongation of the peak latency of the oscillatory potentials on electroretinogram in streptozotocin-induced diabetic rats was prevented with the angiotensin receptor blocker olmesartan, with the agent decreasing neovascularization in mice with oxygen-induced retinopathy.

Clinical retinopathy studies

In a clinical study, Coney et al. (abstract 882) analyzed 368 persons with type 1 diabetes for at least 50 years in the Joslin Diabetes Center registry, 234 of whom were living, with mean HbA_{1c} 7.38%, retinopathy present in 44%, half having required laser treatment, with those reporting retinopathy 1.6- and 2.2-fold more likely to report cardiac and renal disease, respectively. Arun et al. (abstract 879) analyzed retinal photographs of 159 persons with type 1 diabetes for >30 years (mean 39), a group that is thought to be protected from further disease, and showed no retinopathy, nonproliferative retinopathy, and proliferative retinopathy in 1998 in 38, 82, and 39 persons, respectively, and with follow-up in 2003 showing progression in 14, 7 of whom had no retinopathy at baseline. Sight-threatening retinopathy developed in seven individuals (three without retinopathy at baseline). Logistic regression analysis showed significant effect of HbA_{1c}, which averaged 8.3% in nonprogressors and 9.5% in progressors, and of diastolic blood pressure (73 vs. 81 mmHg, respectively), suggesting important modifiable risk factors. The same group (Arun and Taylor [abstract 880]) studied 59 women with type 1 diabetes who had retinal photographs before pregnancy, with 43 and 15 having no and nonproliferative retinopathy, respectively, and 1 having had laser treatment. Retinopathy progressed during pregnancy in 11 (4 requiring laser therapy within a year of pregnancy), with follow-up for 5 years after pregnancy showing nonproliferative retinopathy to be present in 21 and HbA_{1c} being 8.0 vs. 9.7% in nonprogressors versus progressors. Rema and Kumar studied 822 persons with type 2 diabetes in Chennai, India, showing retinopathy to be associated with age, duration of diabetes, systolic blood pressure, lower BMI, fasting plasma glucose, HbA_{1c}, triglycerides, LDL and non-HDL cholesterol, creatinine, and albuminuria, with non-HDL cholesterol remaining significant after adjusting for age, sex, and diabetes duration. Emanuele et al. (abstract 885) reported analyses of baseline fundus photos of 1,210 persons enrolled in the Veterans Affairs Diabetes Trial, reporting worse retinopathy scores to be associated with disease duration, microalbuminuria, and increasing HbA_{1c} and systolic blood pressure, without effect of diastolic blood pressure, lipids, fibrinogen, or plasminogen activator inhibitor-1. Hispanics and African Americans had 1.9- and 1.6-fold higher severe retinopathy prevalence, respectively, which could not be attributed to any of these risk factors. Hallman et al. (abstract 886) evaluated retinopathy in 656 Mexican Americans with type 2 diabetes from 282 sibships that had at least two type 2 diabetic members. A total of 457 persons had retinopathy, and there was no difference in retinopathy prevalence among those whose diabetic siblings had and did not have retinopathy. There was, however, evidence of a familial component to retinopathy severity. Sailesh et al. (abstract 896) studied risk factors for diabetic maculopathy in 629 persons with diabetes. Among 520 having older onset, systolic and diastolic blood pressure and retinal perfusion pressure, but not pulse pressure and cholesterol, were both associated with macular exudates and macular edema patterns, while among 149 with younger-onset diabetes, systolic blood pressure, pulse pressure, and retinal perfusion pressure, but not diastolic blood pressure, were associated with maculopathy, with higher pulse pressure in those with macular exudates than in those with macular edema, suggesting hemodynamic correlates.

Autonomic neuropathy

Aaron Vinik (Norfolk, VA) gave an overview of aspects of autonomic neuropathy, which he noted is present in 50% of persons with diabetes. He therefore recommended screening with heart rate variability measurement, particularly in persons with unexplained syncope, tachycardia, or bradycardia. Erectile dysfunction, orthostatic hypotension, and gastroparesis are characteristic findings. Vinik noted that there is abnormal distribution of cardiac sympathetic innervation in cardiac autonomic neuropathy (CAN), so that even in the absence of ischemia, there may be imbalance leading to arrhythmia. A marker is prolongation of the QT interval, with a level exceeding 430 ms being evidence of neuropathy and a risk factor for arrhythmia. Symptoms of CAN include cough, nausea, dyspnea, and fatigue, with CAN associated with a 2.6-fold increase in the risk of sudden death risk (1). CAN is associated with future deterioration in renal function, and there is evidence for benefit of ACE inhibitor and of β-blocker treatment. Another

aspect of autonomic neuropathy is diabetic small-fiber neuropathy, with the pain of C-fiber type being a burning, superficial allodynia, initially with hyperesthesia and hyperalgesia and later with hypoesthesia and hypoalgesia, although physical examination and nerve conduction may be normal. These patients may be subject to hyperthermia due to a defect in sympathetic thermoregulation and sweating and may have increased risk of foot ulcer and limb loss. The neuropathy is associated with abnormal gravitational blood flow redistribution and correlates with systolic hypertension and low HDL cholesterol. Therapy includes supervised exercise, caution with heat exposure, and avoidance of dehydration, as well as the need for scrupulous foot care and avoidance of hypoglycemia. If patients with small-fiber neuropathy have surgery, there is need to alert the anesthesiologist preoperatively and to watch for arrhythmia. Treatment includes ACE inhibitors and β-blockers, anticholinergics, exercise, and possibly ALA. Heart rate variability improves with glycemic control (2), ACE inhibitors (3), spironolactone (4), and possibly with aldose reductase inhibitors (5). In the Steno-2 Study, an aggressive multifactorial approach to treatment of type 2 diabetes decreased development of autonomic neuropathy by 68% (6). For treatment of orthostatic hypotension, mitodrine and fludrocortisone may be helpful but may aggravate supine hypertension. Vinik suggested that the anticholinesterase pyridostigmine used for myasthenia gravis may offer an additional approach. Future approaches to treatment may include advanced glycation end product breakers, inhibitors of degradation of *N*-acetylaspartylglutamate, protein kinase C (PKC) inhibitors, antioxidants, aldose reductase inhibitors, and neurotrophic drugs.

Several research presentations discussed aspects of autonomic neuropathy. Ziegler et al. (abstract 245) followed 1,801 and 186 persons aged 55–74 years without and with diabetes, with 13 and 32% mortality over 9 years, respectively, showing that a corrected QT interval exceeding 440 ms on the baseline electrocardiogram was associated with doubling of mortality among those without diabetes and with tripling of mortality among those with diabetes. Brown et al. (abstract 966) reported urinary incontinence at least once weekly in 31% of a group of 374 well-functioning older women (mean age 76 years), with incidence more than doubled in association with symptoms of numbness, tingling, or pain in legs or feet. Caucasian ethnicity and obesity were additional risk factors. The Diabetes Prevention Program Research Group (abstract 983) reported urinary incontinence at least once weekly in 44% of 1987 female participants, 39% of those in the lifestyle intervention versus 46% of those in the placebo group, without effect of metformin.

Eckerling et al. (abstract 14-LB) reported efficacy of the GES system Enterra gastric electrical stimulator in 17 type 1 diabetic patients with gastroparesis and medically refractory nausea and vomiting for a mean of 30 months. Six subjects required total parenteral nutrition and four gastrostomy enteral feeding. Fifteen patients no longer required antiemetic medications, with none requiring nutritional support 3 months after the initiation of treatment. BMI increased from 22 to 24 kg/m2 at 4 months, and the gastric emptying rate more than doubled after 6 weeks. In a review of outcome of use of the device in 60 diabetic patients, McCallum et al. (abstract 867) reported improvement in nausea, vomiting, and total symptom score; decrease in hospitalization from a mean of 68 days during the year before the implantation to 19 days during the subsequent year; and decrease in HbA_{1c} from 9.3 to 8.4% at 1 year. Kipnes et al. (abstract 558) treated 60 individuals with diabetic gastroparesis with a macrolide motilin receptor agonist, mitemcinal. After a 28-day course of treatment, gastrointestinal symptoms required discontinuation in five patients, hypoglycemia occurred with increased frequency, and elevated hepatic enzymes were seen, suggesting that this may not provide an optimal approach.

Somatic neuropathy

Phillip A. Low (Rochester, MN) discussed somatic neuropathies, noting that these may involve abnormality of the long peripheral nerve axon, of Schwann cells, of large myelinated fibers responsible for motor and certain sensory functions, of small myelinated fibers responsible for pain and temperature sensation, or of small unmyelinated fiber conveying pain sensation and involved in autonomic function. Thus, diabetic neuropathy may involve sensory fibers causing distal sensory neuropathy, small fibers either diffusely causing autonomic neuropathy or distally causing small-fiber neuropathy, proximal large fibers causing LS radicular plexus neuropathy or proximal polyradiculopathies, single mononeuropathy (ulnar nerve, Bell's palsy, or carpal tunnel syndrome), or involvement of multiple nerve trunks causing mononeuropathy multiplex. Chronic inflammatory demyelinating polyradiculopathy may be immune and require intravenous γ-globulin treatment. The diagnosis of the various forms of neuropathy requires awareness of the differing manifestations of these abnormalities, with sensory neuropathy causing numbness as a late manifestation and excessive function with prickling, stabbing, or burning symptoms characteristically seen earlier. Motor neuropathy leads to weakness and wasting, typically found late in the clinical course and usually less severe as clinical manifestations than the sensory abnormalities. Autonomic neuropathy can lead to vascular effects, including coldness and discoloration, or sweat gland abnormality causing either hyper- or hypohydrosis. Distal sensory neuropathy is commonly associated with dysautonomia and coldness. Nerve conduction measurement is more sensitive than the clinical scores that have been developed, with the Rochester study showing that of 100 persons with diabetic neuropathy, sensitivity was 49% for vibration and 37% for light touch (7). Sural nerve biopsy in patients with lumbosacral radiculoplexus neuropathy shows an intense inflammatory infiltrate, which Low characterized as "spitting out cytokines," explaining the frequent clinical response to steroid therapy.

Mechanisms of hyperglycemia causing neuropathy include abnormalities of the polyol pathway, ischemia, protein glycation, growth factor deficiency, and PKC, all acting via oxidative stress. 8-hydroxy-2[prime]-deoxyguanosine, a marker of oxidative damage to DNA, shows positive immunostaining in Schwann cells in persons with diabetic neuropathy. Further evidence of the inflammatory response is increased nuclear factor κB in Schwann cells and in neural microvessels. There may also be a component of nerve ischemia, with reduced nerve blood flow in experimental diabetes, which can be normalized by ALA in a dose-dependent fashion. A 4-year clinical study of ALA is in progress. Addressing other approaches to treatment, Low sug-

gested that neuropathic pain often responds to local measures, stating that cooling "works at least as well as many medications." He suggested use of the anticonvulsants gabapentin and carbamazepine if pharmacologic treatment is required for painful neuropathy.

A number of studies presented at the ADA meeting addressed aspects of the etiology and potential approaches to treatment of diabetic neuropathy. Obrosova et al. (abstract 142) showed that neural nitrotyrosine, a marker of peroxynitrite-induced injury, and poly(ADP-ribose) accumulation, seen with oxidative stress, are elevated in two rodent models of type 1 diabetes, with FP15, a catalyst for peroxynitrite decomposition, correcting motor and sensory nerve conduction defects and reducing pain sensitivity, suggesting a role nitrosative stress in diabetic neuropathy. Pop-Busui et al. (abstract 141) used a mouse model not expressing COX (cyclooxygenase)-2, showing no improvement in glucose levels in streptozotocin-induced diabetic rats but improvement in nerve conduction and in markers of lipid peroxidation. Uehara et al. (abstract 143) found that diabetic mice had decreased PKC-α levels in dorsal root ganglia, with further decrease in transgenic diabetic animals overexpressing aldose reductase. Administration of an aldose reductase inhibitor increased PKC levels in the transgenic animals but did not improve the abnormality seen in diabetic mice with normal aldose reductase expression. Bril and Buchanan (abstract 851) reported that administration of a new aldose reductase inhibitor, AS-3201 (Dainippon Pharmaceutical, Osaka, Japan) for 12 weeks decreased nerve sorbitol in a dose-dependent fashion, displaying trends to improved nerve conduction velocity and improvement in clinical neuropathy. AS-3201 penetrates sural nerve and inhibits sorbitol and fructose accumulation in patients with diabetic peripheral sensorimotor polyneuropathy.

Kamiya et al. (abstract 144) showed evidence of beneficial effect of C-peptide replacement on small-fiber polyneuropathy in a type 1 diabetic rat model, reducing the hyperalgesic response to heat injury and improving histologic changes of neuropathy, potentially reflecting insulin-like neuroprotective effects of C-peptide and suggesting that hyperglycemia alone is not the only mediator of diabetic neuropathy in this model. Li et al. (abstract 145) reported that a taurine supplemented diet improved nerve function and decreased thermal hyperalgesia in streptozotocin-induced diabetic rats, with evidence of a role of the amino acid in the regulation of neuronal calcium signaling. Nakae et al. (abstract 146) systemically administered basic fibroblast growth factor, which stimulates angiogenesis and cellular proliferation, to streptozotocin-diabetic rats, showing improvement in nerve conduction velocity and in sciatic nerve blood flow but no change in retinal blood flow, suggesting a potential therapeutic role in the treatment of diabetic neuropathy. Naruse et al. (abstract 147) gave bone marrow–derived mononuclear cells to streptozotocin-induced diabetic rats, showing improvement in nerve conduction velocity and sciatic nerve blood flow. Cameron et al. (abstract 148) administered rats the vitamin B_1 analog, benfotiamine, which may decrease accumulation of sugar phosphates, leading to decrease in advanced glycation end product formation. Reduction in endoneurial blood flow and in nerve conduction velocity by streptozotocin-induced diabetes improved, with vasodilatory effects appearing to particularly involve an increase in nitric oxide generation.

Selvarajah et al. (abstract 873) reported abnormality on magnetic resonance imaging of the spinal cord and postero-lateral thalamus in persons with diabetic neuropathy, and Musen et al. (abstract 247) found decreased brain gray matter density in persons with type 1 diabetes without evidence of complications, suggesting involvement of the central as well as peripheral nervous system to be common. Musen et al. further noted an association between hypoglycemia and abnormality of the left temporal gyrus, suggesting that recurrent hypoglycemia as well as hyperglycemia may contribute to these abnormalities. In a study of the long-term benefit of glycemic control, Martin et al. (abstract 244) reported a 36% reduction in the risk of neuropathy at 8-year post-DCCT (Diabetes Control and Complications Trial) follow-up of persons with type 1 diabetes who had had intensive versus conventional glycemic control. Among men, Schade et al. (abstract 872) reported erectile dysfunction in 4 vs. 6% at the conclusion of the DCCT and in 8 vs. 17% 5 years after and 15 vs. 25% 9 years after the conclusion of the trial.

Gore et al. (abstract 531) studied 255 persons with painful diabetic peripheral neuropathy (mean age 61 years, diabetes for 12 years with pain symptoms for 6 years) and found that ~75% of patients had moderate to severe pain symptoms. Forty-seven percent of patients used nonsteroidal anti-inflammatory drugs, 43% opioids, 27% anticonvulsants, 18% second-geneation antidepressants, and 11% tricyclic antidepressants. Mean daily doses were 43 mg amitryptiline and 1,147 mg gabapentin, and pain scores were high with these treatments, suggesting relatively low efficacy. Less than one-quarter of patients reported medications to be extremely or very effective, suggesting high levels of unmet need. Taylor et al. (abstract 621) used a mouse with a point mutation in the α2-δ protein, a subunit of voltage-gated calcium channels, to show that the analgesic and anxiolytic-like actions of pregabalin involve its binding to this site. Strojek et al. (abstract 105) randomized 249 persons with painful diabetic neuropathy and 89 with postherpetic neuralgia to pregabalin 600 mg daily, to 150–600 mg daily with weekly adjustments based on response and tolerability, or to placebo for 12 weeks, with 52, 48, and 24% showing reduction in pain, respectively, but 21, 12, and 8% withdrawing because of adverse events, including dizziness, peripheral edema, weight gain, and somnolence, suggesting that treatment could be optimized by frequent dose adjustment. Portenoy et al. (abstract 599) reported open-label follow-up of 217 patients treated with pregabalin for at least 1 year, showing consistent visual analog pain scores with doses ranging from 75 to 600 mg/day.

Wernicke et al. (abstract 104) examined duloxetine, a reuptake inhibitor of both serotonin and norepinephrine, in the treatment of 791 persons with diabetic neuropathic pain for 12 weeks. Doses of 60 mg once and twice daily were effective after 1 week, although 20 mg once daily was not effective. Discontinuation because of adverse events was seen in <20% of patients. Gozani et al. (abstract 532) reported no significant changes in ulnar or peroneal nerve function with duloxetine 60 mg once or twice daily. In a 9-week study reported by Andorn et al. (abstract 496) of 244 persons with major depressive disorder treated with duloxetine 60 mg daily vs. 251 receiving placebo, 63 vs. 35% showed response and 43 vs. 21% remission, with significantly greater improvement in

overall pain severity, although 13.1 vs. 2.5% discontinued treatment because of adverse events including nausea, dry mouth, and headache. Raskin et al. (abstract 601) assessed glycemic control in a 28-week open-label study of 334 persons with diabetic neuropathic pain treated with 60 mg duloxetine twice daily, reporting a 32-mg/dl increase in fasting glucose accompanied by increase in HbA_{1c}, although noting that this was not seen in 115 persons receiving duloxetine 120 mg once daily. Thirty-six percent of patients failed to complete the study, again suggesting adverse events not to be uncommon. Bosi et al. (abstract 850) used transcutaneous electrical stimuli with sequences of high voltage and low pulse duration in 34 persons with painful diabetic neuopathy and reduced nerve conduction velocity, reporting a reduction in pain, an increase in motor nerve conduction velocity, and a decrease in vibration perception threshold.

The diabetic foot

At a symposium on the diabetic foot, Christopher J. Kwolek (Boston, MA) discussed endovascular treatment of peripheral vascular disease (PVD), suggesting that this may be appropriate for treatment at earlier stages in the spectrum of disease proceeding from claudication to rest pain and from ulcer to gangrene. The term "claudication," he noted, derives from the Latin root for limping or lameness and describes a syndrome of weakness or onset of pain after walking is begun. Claudication can be relieved after a period of rest and may occur during the night because gravity may increase perfusion when upright. Patients with PVD have a high 10-year mortality (48% for those with claudication, 80% for those with rest pain, and 95% for those with gangrene). Signs of peripheral ischemia include absent pulses, blanching on elevation, dependent rubor, delayed venous filling, shiny atrophic skin, loss of hair on lower extremities, and, ultimately, atrophy of the subcutaneous tissues and progression to gangrene. Noninvasive modalities include the ankle-brachial index (ABI), with claudication typically at levels <0.6 and rest pain <0.5, although arterial wall calcification may cause noncompressible vessels, leading to a higher ABI despite significant arterial disease. Tobacco use, hyperlipidemia, and diabetes are risk factors. Medical management includes a walking program, smoking cessation, risk factor modification, antiplatelet therapy with aspirin and clopidogrel, anticoagulation with warfarin, and the drugs pentoxifylline and cilostazol, which offer symptomatic relief.

Goals of revascularization are the relief of lifestyle limiting claudication and limb preservation. It is crucial to assure that there is adequate inflow from the aortoiliac vessels, where percutaneous interventions, including percutaneous transluminal angioplasty (PTA) and stenting, are now viable alternatives to open surgical procedures, with initial technical success in 95%. Although Kwolek acknowledged that 2-year patency for extensive external iliac disease may be only 45% and that overall 5-year patency rates are ~40% (half of those reported with open bypass), he noted that newer data suggest that PTA outcome is improving. With repeat intervention, results are excellent and patency may be as high as 80%. Furthermore, PTA/stenting is less invasive, decreasing duration of hospitalization and leading to quicker recovery. The approach leads to less morbidity in terms of leg swelling and loss of ambulatory capacity after procedure than with surgery. Potential complications include dissection, embolization, thrombosis, pseudoaneursym, rupture, the need for repeat procedures, and difficulty with later intervention. Endovascular intervention is particularly useful for shorter lesions but is now being attempted with longer and multiple lesions. Combination procedures may be useful with proximal percutaneous endovascular intervention and distal bypass. Kwolek stressed the need to individualize treatment based on the patient's age, comorbidities, and anatomic characteristics, and he mentioned promising new technologies, including coated stents, drug-eluting stents, vascular brachytherapy (localized radiation therapy), cryotherapy, gene therapy, rotablators (miniature drills capped with an abrasive, diamond-studded burr), and lasers. A large number of self-expanding stents are now available, most of which are approved for hepatobiliary disease and are used "off-label," with reported technical success rates in PVD of 98%, and 76 and 60% patency at 12 and 24 months, respectively. In his series, >50% of patients had greater than three lesions treated, with low postprocedure morbidity and no mortality.

Boulos Toursarkissian (San Antonio, TX) discussed surgical revascularization of the diabetic foot. He noted that the diabetic foot ulceration affects 15–20% of persons with diabetes during their lifetime, caused by neuropathy, ischemia, infection, and microvascular disease, the latter including thickened basement membranes, arteriovenous shunting, decreased vasodilation, and abnormal endothelial function with decreased angiogenesis. A significant percentage of diabetic patients have PVD, often affecting vessels below the knee, although he suggested that "the mere presence of PVD is not necessarily an indication for intervention." Revascularization does not reverse neuropathy, and indications for intervention are rest pain, ulcer, nonhealing wounds, or requirement for surgical intervention, which would not be likely to heal without restoration of arterial inflow. There is no single noninvasive parameter that will reliably predict healing, and a palpable pulse does not always mean normal circulation, with Toursarkissian suggesting that one should "err on the side of revascularization" unless the patient is a prohibitive risk, has poor target vessels, or has a minimal ulcer. Older studies suggest that revascularization should be done for ankle systolic pressure ≤40 mmHg, but Toursarkissian recommended intervention at levels ≤70 mmHg. An alternative measure is toe pressure; 80% of wounds heal with levels >40 mmHg, with levels ≥60 mmHg desirable.

Revascularization may be performed by endovascular approaches, with angioplasty, stenting, thrombolysis, and atherectomy or with an open-surgical approach such as endarterectomy. Often, both approaches are used together. Infrageniculate (below knee) arterial occlusion occurs more frequently than stenosis, typically with relative sparing of the foot. Any vessel relatively free of stenosis may provide inflow, using as short a bypass as possible. The best conduit is the ispilateral greater saphenous vein, with alternatives including the lesser saphenous vein and arm veins, although the likelihood of good healing is at least 10–20% lower with these approaches. The use of the contralateral greater saphenous vein is controversial as a bypass of the other leg is needed in 50–60% of patients over the subsequent 5 years, and nonhealing of the graft harvest site may be a complication. Alternatives include cryopreserved veins and prosthetic polytetraflouroethylene for tibial bypasses. Arterial autografts using subscapular artery or deep inferior

epigastric artery are being studied as options. The best outflow is a vessel that is axially continuous to the foot and relatively free of stenosis, with size not being crucial unless the vessel is both small and diseased. The peroneal artery is not as good as the posterior tibial or dorsalis pedis arteries in the foot, with the dorsalis pedis not as good as the posterior tibial if a transmetatarsal amputation is planned. In situ grafts are preferred when the vein diameter is <2–3 mm distally. Technical adjuncts include "skip incisions" rather than one continuous incision to avoid flap necrosis and wound healing problems, vein mapping by duplex Doppler testing before surgery, and the use of clamps which do not damage the vein. Anticoagulation appears to improve outcome. Results of bypass surgery vary with the experience of the surgeon and with the level of the bypass, with one study reporting 80, 70, and 57% patency at calf, dorsalis pedis, and tarsal or plantar level, respectively. Results also vary with the conduit, as arm vein grafts have 40–50% patency while lesser saphenous vein grafts have 50–60% patency at 3–5 years, with limb salvage rates usually 10–15% better than patency rates. There is evidence of lower limb salvage after revascularization in persons with diabetes, but most studies do not show differences in patency rates, with a 5-year study of 962 vein grafts showing 83% limb salvage in diabetic patients, and Toursarkissian's center having 78% patency and 71% limb salvage rates. Bypass complications include 1–2% mortality, 1–2% risk of bleeding, and 5–7% rate of thrombosis within 30 days, with wound healing problems and edema almost universal. For infrageniculate bypass, failure rates are ~30 vs. 10% for poor versus good angiographic appearance scores with corresponding likelihood of limb salvage. Diabetic patients on dialysis may have acceptable patency rates but lower-limb salvage rates, with one-year limb salvage rates 50–60%, and with poor patient survival. Infection is not a contraindication for bypass as long as the bypass incision is away from the site of infection.

Late failure of bypass is another issue, with 15–20% developing stenosis, a complication not occurring more frequently in persons with diabetes. Grafts revised before thrombosis have better patency rates than those revised after this has occurred. Once a graft has stenosed once, stenosis is likely to recur even following successful surgical correction. Up to 50% of diabetic patients who occlude a graft placed for limb salvage are able to tolerate its loss after the initial insult (ulcer or infection) has resolved, although Toursarkissian noted that early failure of a graft done for ulceration appears to be a particularly bad sign. Adverse outcome is also associated with heart failure, with nonambulatory patient status, with gangrene as the initial bypass indication, and particularly with the continuing presence of a foot ulcer. Patency of secondary reconstruction is less than with primary reconstructions, particularly with artificial grafts, and only the minority of persons with failed grafts are able to undergo a second procedure or throbmolysis, so that "the first bypass is your best bet," and ongoing duplex surveillance every 3 months during the first year should be done.

Ronald Sage (Maywood, IL) discussed the long-term results of limb salvage amputations, including transtibial/transfemoral and partial foot, the latter constituting approximately half of amputations performed in the U.S. Indications include uncontrolled infection, intractable pain, necrosis of bone or tendon, and, sometimes, debilitation for patients who are unable to tolerate efforts at rehabilitating the nonamputated extremity. Limb salvage and quality of life issues need to be addressed, Sage stated, with evidence that limb salvage patients report a decline in physical function and general health (8) and that early rehabilitation after amputation may promote better outcome than limb salvage. Transtibial amputees, Sage said, have no difference in health perceptions from matched patients. Persons with foot deformity and chronic ulcers typically have neuropathy rather than vascular disease as their major problem, although the contribution of PVD is shown by a series of 692 digital amputations reported >30 years ago; of 365 individuals with palpable pulses, there was only a 2.2% failure rate, while 277 without palpable pulses had an 11% failure rate (9). Fifteen percent of diabetic patients ulcerate during their lifetime, with "Pecoraro's Triad" of minor trauma, ulceration, and faulty healing (10) the typical causes. Wound healing success depends on infection control, glycemic control, adequate vascular supply (with, for example, an ABI >0.45 or transcutaneous O_2 pressure >30 mmHg), serum albumin >2.5–3.0 (a nutritional indicator), overall medical (in particular, cardiac and renal) status, and prevention of further abnormal foot pressure with offloading.

Partial foot amputations include toe, ray, midfoot, and Synes amputations. Ray resection is indicated for nonhealing ulcer of a toe or at a metatarsal bone, although it is often only a preliminary to higher surgical resection (11). Transmetatarsal amputation, performed in appropriate patients, may lead to ulcer healing in more than three-quarters of patients (12). Higher amputations through the midfoot are the Lisfranc amputation at the tarsometatarsal joints and the Chopart amputation at the midtarsal joints, both of which result in the development of equinovarus deformity, requiring lengthening of the Achilles tendon, and only approximately half of these amputations heal without complications (13). Sage noted the poor prognosis of heel ulceration and the frequency of heel ulcers appearing on the contralateral foot after extensive surgical treatment of a foot ulcer. Syme's amputation is, he stated, often useful (14), and although healing is delayed with the older two-stage approach, it is now performed in one stage (15), with more than three-quarters of patients healing and able to ambulate. The poor long-term patient survival may be a particular rationale for use of amputations to allow more rapid rehabilitation, and the Symes amputation leads to ambulation requiring less exertion than with higher-level procedures, improving the likelihood of a good functional result. Better body image and ability to ambulate in emergency may be additional reasons to use this approach more widely than higher amputations, which Sage suggested be reserved for persons with uncontrolled infection, vascular disease, poor cardiac and renal status, and/or malnutrition. Outpatient treatment is, he said, crucial, including use of protective shoes, frequent debridement of keratoses and ulcers, and patient education, recognizing that patients who have had an ulcer are at 13× increased risk of subsequent ulcer. Sage concluded, "There is no such thing as routine care for diabetic feet."

In a study presented at the meeting, Tierney et al. (abstract 171) presented analyses from the Centers for Disease Control database of lower-extremity disease in diabetes in the U.S., reporting that

lower-extremity amputations occurred annually in 0.65% of persons with diabetes, with ~4% of persons with diabetes hospitalized annually for peripheral arterial disease, 4% for neuropathy, and 3% for ulcer or infection (presumably with overlap between these groups), with 12% reporting a history of a foot sore within the previous year. Almost one-third of persons with diabetes reported not receiving a foot exam in the previous year. Among 218,528 persons with diabetes treated in the Veteran's Hospital system, Tseng et al. (abstract 172) reported that 1.4% had amputations annually. Edmonds and Foster (abstract 173) assessed the relationship between diabetic neuropathy and distal arterial disease in amputated limbs of 38 diabetic and 34 nondiabetic patients, showing a correlation of neuropathy with calcification and plaque formation, although not with thrombosis, suggesting the potential for a causal relationship. Katz et al. (abstract 176) reported that a "removable cast walker" could be made nonremovable by wrapping it with a single strip of fiberglass cast material and showed that with this approach to ulcer healing occurred as rapidly (4–5 weeks) as with total contact cast application in 38 persons with noninfected, nonischemic plantar neuropathic foot ulcers that had been present for a mean of 220 days before the study. They suggest that this approach could "make effective offloading universally available without the need for highly skilled technicians." Petrova et al. (abstract 177) reported that quantitative ultrasound of both ankles of persons with acute unilateral Charcot osteoarthropathy showed reduction in bone mineral density to occur with the disease, with evidence of preexisting ostopenia in persons with type 1 but not type 2 diabetes, suggesting benefit of treatment with antiresorptive agents. Ruotolo et al. (abstract 178) reported a randomized controlled trial of use of the bisphosphonate alendronate in 18 persons at the onset of acute Charcot neuroarthropathy, showing an increase in bone density of the foot and reduced levels of markers of bone reabsorption.

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