National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) meeting summary
Advances toward measuring diabetic retinopathy and neuropathy: from the bench to the clinic and back again (April 4–5, 2007, Baltimore, Maryland)

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Abstract

The National Institute of Diabetes and Digestive and Kidney Diseases sponsored a meeting recently to explore new ways to assess diabetic retinopathy and neuropathy, both in diabetic patients and in diabetic mice. The workshop compared current gold standards for assessment of retinopathy and neuropathy, new improvements of existing techniques, and new functional biomarkers measured with nontraditional technologies. Since the anatomical changes that comprise diabetic retinopathy and neuropathy take long to develop and have proven difficult to arrest once initiated, some talks highlighted the value of methods that are based on the pathophysiology that precedes, and might contribute to, the histopathology. In addition, a goal of the workshop was to produce a set of working criteria on phenotyping diabetic retinopathy and neuropathy that could be reviewed by the scientific community.

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1. Introduction

On April 4–5, 2007, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) sponsored a workshop to explore the current criteria for the diagnosis of diabetic retinopathy and neuropathy in man and how these evaluations compare with the current measures used to define these complications in animal models. The goal of the NIDDK workshop was to begin a dialogue that will lead to an accepted set of optimized working criteria for phenotyping and defining retinopathy and neuropathy in rodent models of diabetes. Day 1 of the workshop reviewed currently accepted standards for diagnosis in man and rodent models as well as new biomarkers and technologies for human and rodent phenotyping. Day 2 focused on cutting-edge technologies, specifically spectroscopy, hydrospectral imaging, and magnetic resonance imaging (MRI). In this meeting summary, discussions on retinopathy and neuropathy are separated, although throughout the workshop, the 75 attendees frequently discussed the interplay of the two complications.

2. Retinopathy

Diabetic retinopathy is the leading cause of vision loss in adults in the USA. Retinopathy is currently characterized by a spectrum of retinal lesions and abnormalities that indicate
vascular damage (capillary microaneurysms, capillary degeneration, vascular permeability, new vessel formation) and death or dysfunction of the neural retina (cotton wool spots, alterations in retinal electrophysiology, color or hue discrimination). Clinically, retinopathy has been separated into nonproliferative and proliferative stages of the disease determined by the assessment of neovascularization or abnormal growth of new retinal blood vessels into the vitreous. Typically, two aspects of diabetic retinopathy, neovascularization and retinal edema, are associated with adverse effects on vision. These abnormalities appear to be dependent on changes that develop in the earlier stages of the disease. Occlusion or degeneration of retinal capillaries is strongly associated with the development and progression of diabetic retinopathy, presumably by contributing to development of ischemia with subsequent release of hypoxia-inducible vasoproliferative factors. Since diabetic retinopathy takes years to develop, and only a fraction of patients will progress to visual impairment from the disease, there are two major questions of clinical significance today: (1) how to identify, earlier in the course of the disease than is now possible, which patients will progress to visual impairment or loss and (2) how to determine the effects of therapies.

In April 2007, about 75 investigators gathered in Baltimore to discuss these two questions as well as the question of the extent to which mice can be a useful model of diabetic retinopathy. The initial two speakers presented current gold standards in methods to assess diabetic retinopathy in both animals and patients. Both speakers emphasized that damage to the retinal microvasculature is currently believed to cause clinically significant retinopathy. Dr. Tim Kern presented an overview of diabetic retinopathy in animals, which focused primarily on the vascular histopathology, but he discussed also the relation between retinal function, neurodegeneration, and the vascular lesions. Histopathology is well established, quantitative, and comparable to information obtained clinically. Available animal models develop vascular lesions characteristic of the early stages of diabetic retinopathy but do not develop the late stages of the retinopathy, including preretinal neovascularization.

Dr. Neil Bressler focused on results of large multicenter clinical trials, including the Diabetic Retinopathy Clinical Research Network (DRCR.net), a collaborative network that facilitates multicenter clinical research on diabetic retinopathy and macular edema. These trials have relied on three principal methods for characterizing the retinopathy [color photos of the retina, stereoscopic slit-lamp examination, and more recently, optical coherence tomography (OCT)] to assess retinal thickness (due to retinal edema)]. The DRCR. net has found that retinal thickening detected via OCT is not associated with altered visual acuity. These data suggest that retinal thickening (presumably caused by excessive permeability of the retinal vasculature) cannot be used to predict visual acuity in all patients.

OCT remains a promising technology that enables noninvasive, high-resolution, cross-sectional imaging of the retina and anterior eye. This allows for quantitative morphometry of retinal architecture, such as retinal thickness or retinal nerve fiber layer thickness, the inner and outer segments of the photoreceptor layers, and retinal pigment epithelium. Dr. James Fujimoto discussed new OCT detection techniques, including spectral/Fourier domain detection that have improved imaging speed and spatial resolution. These advances may enhance current efforts to identify diabetic retinopathy, both in diabetic patients and animals. Functional OCT techniques are being developed that may enable integrated structural and functional imaging, although at present, the physiology underlying the functional changes is not clear.

Dr. Anthony Adams and Marcus Bearse, Jr., summarized their work using multifocal electoretinogram (mfERG) to evaluate retinal function in multiple sites across the retina. They find that focal delays in latency detected by mfERG can predict the retinal locations of new nonproliferative diabetic retinopathy (i.e., microaneurysms) development over 1- and 2-year periods and that the delays become increasingly abnormal as local retinopathy status worsens. These data suggest that mfERG may be useful in predicting specific locations of pathology in the retina. The application of mfERG to mice is limited by retinal size at this time.

Dr. Mara Lorenzi focused on biomarkers of early diabetic retinopathy, especially acellular nonperfused capillaries, macular edema, and activation of retinal Müller cells. Additional studies report that decreased retinal blood speed precedes evidence of diabetic retinopathy and might foretell the closure of retinal capillaries that leads to retinal ischemia and eventually triggers proliferative retinopathy. Recent studies of hemodynamics in diabetic patients suggest that altered caliber of retinal arterioles and/or venules can predict incidence or progression of clinical retinopathy. Understanding the natural history and mechanisms of the abnormal reactivity of retinal vessels, even in well-controlled diabetes, could yield important early markers of retinal microangiopathy.

The remaining speakers focused their talks on new techniques that might offer new opportunities for pheno-
typing diabetic retinopathy. These talks highlighted the value of methods that are not based on anatomical changes (when the retinopathy is more difficult to arrest) but on the potentially reversible pathophysiology that precedes anatomical changes.

Dr. Bruce Berkowitz reported that significant perturba-
tions of MRI measures of retinal oxygenation and apparent ion demand (measured by manganese uptake) appear prior to retinal lesions in diabetic rodents. Supernormal retinal oxygenation is also found in patients with diabetes even before clinical retinopathy is detected. MRI is a noninvasive, spatially and physiologically accurate in vivo method that can be used to simultaneously collect prognostic retinal histological and physiological information in animal models.
and clinical trials. Importantly, drug treatments that correct functional MRI metrics predict later inhibition of histopathology, while therapies that do not correct abnormalities correlate with lack of inhibition of histopathology. Dr. Berkowitz noted that imaging of such pathophysiologic biomarkers during the clinically silent phase of diabetes is a key to the future of drug development. Such functional MRI metrics will provide answers for critical clinical questions concerning which drug target, dose, and schedule are most likely to be effective.

Dr. Kurt Denninghoff covered the discovery and validation of a new technique for noninvasive monitoring of abnormal retinal vessel autoregulation, an early indicator of risk in diabetic retinopathy, using retinal hemoglobin oxygen saturation measurements. In animal and human studies, oxygen autoregulation and delivery to the retina are abnormal in diabetic retinopathy. These changes in autoregulation occur prior to anatomical changes in the retinal vasculature. In agreement with the work by Drs. Lorenzi and Berkowitz, he suggested that abnormal autoregulation might be an early indicator of risk of developing diabetic retinopathy. A number of eye oximetry devices noninvasively estimate the amount of oxygen reaching the brain by measuring the saturation level of the blood in the retina through the use of a spectroscope. Previous calibrated measurements have been difficult to obtain due to inaccurate extinction coefficients, uncontrolled variables (including light path, pH, and hematocrit), tissue and blood cell scattering, wavelength selection, and inaccurate baseline assumptions. The new approach described by him appears to adequately address these concerns.

In summary, mice currently offer several advantages to study the pathogenesis of diabetic retinopathy, most notably the ability to genetically manipulate these animals and their low cost. Some of the histopathology changes that are characteristic of diabetic retinopathy in humans (e.g., neovascularization) have not been reported so far in diabetic mice. Nonetheless, diabetic mice do develop abnormal morphology that is consistent with the earliest histopathology associated with diabetic retinopathy as well as apparently clinically relevant changes in retinal neural function, vascular autoregulation, retinal thickness, and vascular permeability. One conclusion of the meeting was that early functional phenotyping of diabetes-induced retinal abnormalities, uncovered by a challenge or kinetic measurement (not in steady state), might complement and extend existing anatomical methods of assessing the disease. Thus, spectroscopy and MRI are clearly useful, and their use should be encouraged as valuable approaches to transitioning between diabetic mice and patients.

3. Neuropathy

Diabetic neuropathy is the most common complication of both types 1 and 2 diabetes. Diabetic neuropathy is characterized by a slowly progressive, length-dependent loss of sensation that correlates with diabetes duration and glycemic control. Patients with diabetic neuropathy relate “positive” symptoms including burning, pain, and paresthesias, or negative symptoms characterized by numbness and lack of pain perception. The meeting turned from discussions of diabetic retinopathy to diabetic neuropathy. Dr. Vera Bril outlined the gold standard for the diagnosis of diabetic neuropathy, accepted by the American Academy of Neurology, the American Association of Neuromuscular and Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. This standard includes the following:

- There should be at least one neuropathic symptom.
- There should be at least one neuropathic sign.
- Abnormality in objective testing should be present, which may include “electrophysiological polyneuropathy” as defined by an abnormality of any nerve conduction parameter in two or more nerves; if both sural and peroneal nerves are normal, diabetic neuropathy is not present.

Dr. Bril emphasized that existing clinical assessment tools and measurement methods produce an accurate diagnosis. Symptoms may be assessed using a visual analogue scale and Likert scale for pain, the multidimensional McGill Pain Questionnaire, or the Neuropathy Total Symptom Score-6. Physical assessments may include the Complete Neuropathy Assessment, Toronto Clinical Neuropathy Score, Total Neuropathy Score, and Michigan Neuropathy Screening Instrument. These tools employ one or more of the following clinical assessments at the great toe: vibration perception threshold, pin prick, pressure, and/or temperature; the presence of reflexes, particularly at the ankle, is assessed and, if absent or depressed, supports the diagnosis of diabetic neuropathy. Since complications are more difficult to treat with disease progression, early diagnosis of diabetic neuropathy in the diabetes clinic is essential to reduce these complications, especially ulcer formation.

Dr. Nigel Calcutt discussed the common rodent models of diabetes, which include the streptozotocin (STZ)/alloxan rat or mouse and the BB rat for type 1 diabetes, and the Zucker obese rats, db/db mice, ob/ob mice, and mice maintained on a high-fat diet for type 2 diabetes. There are advantages and disadvantages to each model for the investigation of rodent diabetic neuropathy. Contributing factors to the neuropathy phenotype in rodents include background strain, diet composition, insulin/C-peptide deficiency, coexisting hyperglycemia and hypertension, and duration of diabetes. Current biomarkers for diabetic neuropathy in diabetic rodents may be functional, behavioral, or structural. Functional biomarkers include nerve conduction slowing and resistance to ischemic conduction block in large fibers, impaired regeneration, and neuropeptide synthesis and transport in small fibers. Behavioral biomarkers include mechanical/chemical
hyperalgesia, tactile allodynia in sensory large fibers, and thermal nociception in sensory small fibers. Structural biomarkers include axonal number/caliber in nerve trunks of large fibers and epidermal fiber numbers/morphology in small fibers.

The next group of speakers presented talks on new biomarkers and technologies in diabetic neuropathy. Dr. Rayez Malik introduced the novel idea of using corneal confocal microscopy (CCM) as a diagnostic tool for quantifying the degree of diabetic neuropathy. CCM is noninvasive and takes only a few minutes in the outpatient office. Studies on CCM show promise for diagnosing and stratifying the severity of diabetic neuropathy. Measures of corneal nerve length, nerve fiber density, nerve branch density, and nerve tortuosity may be assessed by CCM. Abnormalities of these parameters by CCM correlate with the degree and severity of diabetic neuropathy, classified by more standard clinical and electrophysiological parameters. In an unpublished study, CCM compares favourably to skin biopsies in quantifying nerve fiber loss, and improvement in nerve function correlates with improved corneal nerve fiber density quantitated by CCM. In conclusion, CCM is a novel, noninvasive in vivo clinical technique that may be used to diagnose and stratify the severity of diabetic neuropathy. CCM could provide a novel method to assess progression and efficacy of new therapies in diabetic neuropathy.

Dr. Michael Polydefkis reviewed the utility of quantitating intraepidermal nerve fiber density (IENF) from skin biopsies as a measure of diabetic neuropathy. Multiple studies have shown that IENF is reduced in the setting of normal sural amplitudes, suggesting that IENF is a more sensitive marker of early disease, particularly in diabetic neuropathy. Routine skin biopsies with assessment of IENF may also be used to identify more homogenous populations for neuropathic pain studies as well as regenerative studies. The power of routine skin biopsies can be expanded by selectively destroying the intraepidermal fibers in a fixed region of the thigh via capsaicin application and monitoring nerve fiber regeneration with repeated skin biopsies of this same area. Using this approach, the regenerative rate of diabetic patients was compared to healthy age-matched controls. Regeneration occurred in a linear fashion at a constant rate through the first 90 days in both patient groups. Between days 90 and 180, there was a decrease in the regenerative rate in the patients with diabetic neuropathy. Healthy controls reached baseline levels by day 180. In contrast, during the same time frame, patients with diabetes reached a maximum level of regeneration at 59% of baseline and then plateaued to 54% of baseline. This suggests that, in the setting of hyperglycemia, even mild recovery is incomplete. The study also validates this novel approach for quantification of nerve regeneration that could be applied as a biomarker to assess the efficacy of pharmacologic interventions in diabetic neuropathy.

Dr. Paul Fernyhough turned the discussion from novel methods used in humans to assess diabetic neuropathy to newly discovered means of assessing damage in animal models of diabetic neuropathy. Aberrant calcium homeostasis is observed in dorsal root ganglion (DRG) sensory neurons in diabetes and may originate in the endoplasmic reticulum (ER). The ER-dependent rise in intracellular calcium in diabetes induces enhanced rates of oxidative phosphorylation by the mitochondria and increased oxidative stress. High extracellular glucose and/or impaired growth factor signaling leads to impaired ER membrane protein function. Abnormal calcium homeostasis in diabetic neurons is characterized by raised resting calcium levels, reduced caffeine-induced release, more severe glucose-mediated effects in the lumbar DRG, and prevention of abnormal calcium homeostasis by neurotrophin-3 or insulin. Calcium uptake and leakage from the ER is impaired at least twofold in diabetes, sarco(endo)plasmic reticulum calcium adenosinetriphosphatase expression is lowered, intracellular calcium is raised, and mitochondria are depolarized. These conditions contribute to impaired axon outgrowth from diabetic neurons, and indeed, mitochondrial oxidative stress is observed to be elevated in diabetic axons. These axons have abnormal swellings filled with adducts of 4-hydronenonal, which disrupts axonal outgrowth. Treatment with the antioxidant N-acetylcysteine lowers levels of reactive oxygen species, reduces abnormal swellings, and improves axon outgrowth. These novel findings can contribute to animal phenotyping for diabetic neuropathy. Dr. Douglas Zochodne reviewed established markers of rodent diabetic neuropathy and introduced a discussion of new disease markers. He emphasized that idea that different models of diabetic neuropathy may be used to ask and answer unique questions. For example, his laboratory has reported diabetic neuropathy in the C57 CD1/Swiss Webster STZ mouse. Diabetic neuropathy was quantitated over time using behavioral assays of sensation and serial nerve conduction studies. Structural indices of neuropathy in this model include axonal atrophy, loss of distal sural axons, early axonal degeneration, and myelin thinning. Dr. Zochodne maintained that molecular measures of diabetic neuropathy may offer the greatest opportunity to understand early mechanisms of neurodegeneration. Several molecular measures have been investigated, including down-regulation of structural proteins (neurofilament, tubulin) and growth factor receptors (Trks, p75) and up-regulation of markers of neurotoxicity, for example, nitrotyrosine, poly (ADP-ribose) polymerase, activated caspase, receptor for advanced glycation endproducts (RAGE) and nuclear factor kappa B (NFκB). Alternations in critical intracellular pathways, including insulin and insulin-like growth factor, have also been studied both in vitro and in rodents with diabetic neuropathy. Dr. Zochodne concluded that understanding relevant molecules and intracellular pathways may help us to better understand why sensory neurons are particularly targeted by diabetes, whereas motor neurons are spared.
4. Concluding remarks

Dr. Eva Feldman thanked the speakers and attendees for their contributions, as well as the NIDDK for its sponsorship of the workshop. She summarized that two key questions were discussed: What criteria should be used to determine that a mouse model of diabetic retinopathy or neuropathy represents a valid model of human disease? Can acceptable paradigms for optimal phenotyping of murine models for diabetic retinopathy and neuropathy be established and translated from mouse to man and back again? The workshop provided a forum for frank discussions, and attendees heard about an array of new technologies available. The current working criteria for phenotyping mouse models of diabetic retinopathy and neuropathy, as well as nephropathy and cardiovascular disease, can be found on the Animal Models of Diabetic Complication Consortium (AMDCC) web site www.amdcc.org. The criteria for retinopathy and neuropathy are considered a “work in progress,” and all meeting attendees as well as individuals interested in diabetic complications are welcome to provide further comments and insights using the AMDCC web page.

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