



Report on the 11th EASD/JDRF Oxford Workshop, 21-24 July, 2006

Regeneration in Nervous System Complications of Diabetes:
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Introduction

Regeneration in diabetes nervous system complications was the topic of the 11th EASD/JDRF Oxford Workshop. This topic was chosen to highlight the potential for new means to maintain and restore function in the brain, peripheral nerve and retina in persons with diabetes.

Diabetes impacts all organs and the impact on the nervous system and leads to substantial clinical morbidity, ranging from stroke, cardiac arrhythmias, gastrointestinal motility disorders, retinopathy, and peripheral neuropathy. In addition, diabetes also has been recognized to impact cognitive function. These complications can be mitigated by intensive metabolic control but, once established, are difficult to reverse with current therapies. Most drug trials for diabetes complications have failed. Current medications for peripheral neuropathy provide only partial symptomatic relief and rarely improve neural function. No pharmacologic therapy is FDA-approved for diabetic retinopathy. Patients with diabetes have worse stroke outcomes than do non-diabetic persons.

Strategies to promote beta cell regeneration in patients with Type 1 diabetes are being tested, but there are currently no established regenerative interventions for diabetes complications. Therefore, researchers were assembled with experience in regeneration following trauma, ischemia and chronic neurodegenerative conditions such as Alzheimer's disease and multiple sclerosis. The potential for successful intervention in diabetes should be higher than these other conditions because of its slower onset and partial impairment compared to trauma or ischemia, and the availability of animal models. Diabetes is chronic and affects world-wide populations to much greater degree than any other disease so there is an enormous market potential. Therefore, it should attract substantial investment of resources.

The meeting followed the established format for EASD/JDRF workshops, with presentations by teams of senior investigators and post-doctoral fellows in several thematic areas. Representatives of JDRF, NIDDK, The Wellcome Trust, and sanofi-aventis also participated. Research topics were presented by senior scientists along with junior personnel from their research laboratories, and these presentations were followed by group discussion including a round table discussion at the end of the meeting.

Meeting participants:

Keynote lecturer: Dame Julia Polak (Imperial College, London)

Senior scientists and Fellows: Thomas Gardner (Chair) and Ravi Singh, Ian Simpson and Rashmi Kumari, David Antonetti, (Penn State College of Medicine), Rayaz Malik, Mitra Tavakoli (University of Manchester), Douglas Zochodne and Cory Toth (University of Calgary), Jeff Goldberg (Bascom Palmer Eye Institute), Guanvito Martino and Stefano Pluchino (San Raffaele Scientific Institute), Kwok-Fai So (Li Ka Shing Medical Facility University of Hong Kong), Geert Jan Biessels and Ineke Brands (University of Utrecht), Colin Barnstable and Joyce Tombran-Tink (Yale University / Penn State College of Medicine), Maria Grant and Sergio Li Calzi (University of Florida), Patrizia Ferretti and Rachael Dobson (University College London), Gerald Schneider and Rutledge Ellis-Behnke (MIT), Michael J Young and Ji Yeon Kim (Schepens Eye Institute, Harvard Medical School), José-Alain Sahel and David Gaucher (S Antoine Hospital, INSERM), Aviva Tolkovsky and Edward Bampton (University of Cambridge/ University of Leicester), John Sinden and Erik Miljan (ReNeuron), Lynn Allen-Hoffman (Stratatech), Christina Thomas-Virnig (Stratatech), Kim Hewitt (Aegera Therapeutics)

Keynote address

Dame Julia Polak (Imperial College London) was the keynote speaker on the role of stem cells and tissue engineering. She presented her extensive experience with embryonic stem cells and discussed the roll of the microenvironment, the potential for the genetic modulation, and the requirements to put stem cells in a suitable environment (1). Most of her work has involved regeneration in lung and bone the principles apply to most tissues.

Scientific sessions

Session 1 examined diabetes as a platform for tissue regeneration and repair.

Douglas Zochodne and Cory Toth (University of Calgary) discussed impaired nerve regeneration in diabetes. Dr Zochodne described a “double hit” approach in preventing neurodegeneration: first the progressive polyneuropathy must be halted; then nerves must be coaxed to resume their original function. Mechanisms of nerve failure include: glucose toxicity, microvascular dysfunction, inflammation (hematogenous macrophage infiltration, nuclear factor kappa B (NF- κ B expression)) decreased sprouting, delayed Wallerian (distal) regenerative programming, retrograde axonal loss, and impaired insulin signaling. He also pointed out that microvascular plasticity is impaired with less vasodilation. There is however decreased myelin and low-grade inflammation with. Treatment of diabetic rats with insulin at the site of nerve injury accelerates the repair process without lowering blood glucose (2). In diabetic db/db mice they find increased expression of the receptor for advanced glycation end products (RAGE) in white matter (brain parts with myelinated nerve cell axons, which connect various grey matter areas (the locations of nerve cell bodies) of the brain to each other and carry nerve impulses between neurons—Wikipedia) compared to age-matched controls (3). Intranasal insulin improves performance on a cognitive test in a water maze in these mice, without lowering blood glucose but does not protect against white matter changes. In summary, these findings indicate that the process of peripheral and central nerve damage in diabetes is complex and normal reparative processes are impaired, and regeneration may be enhanced by controlling the adaptive process.

Session 2 focused on the immune, metabolic, signaling and growth factor aspects of nervous tissue regeneration.

Jeff Goldberg (Bascom Palmer Eye Institute) presented his work on the survival and regeneration of retinal ganglion cells (4). He pointed out that trophic responsiveness in optic nerve cells is rescued by increased cyclic AMP and by depolarization. Retinal ganglion cells have a calcium-dependent adenylate cyclase and its removal abrogates effects of trophic factors. During embryonic development, amacrine cells in the retina turn off retinal ganglion cells to inhibit axonal growth but increase dendrite activity. Understanding the mechanisms of ganglion cell growth and differentiation may provide clues to stimulate regeneration.

Guanvito Martino and Stefano Pluchino (San Raffaele Scientific Institute) discussed the potential role of neural stem cells for therapy of brain disorders (5). Brain inflammation occurs as part of central nervous system (CNS) disorders such as Parkinson’s disease and multiple sclerosis; ultimately, macrophages destroy myelin. Three components of regeneration include: control of inflammation, cell plasticity to recruit non-damaged neuronal pathways, and oligodendrogenesis. Neural stem cells are found in the hippocampus and sub-ventricular zone, are expandable, and can cross the blood-brain barrier. Neural stem cells have an immune signature with constitutively clustered integrins and other surface markers. Neural stem cells appear to work via bystander effects rather than directly replacing deficient cells. These cells appear to have neurotrophic effects on existing cells. Important questions remain about optimal routes of delivery and means to modulate the immune interactions for optimal effects.

Rayaz Malik and Mitra Tavakoli (University of Manchester) discussed the use of corneal confocal microscopy as a non-invasive surrogate marker for human diabetic neuropathy (6). They emphasized that the many clinical trials of diabetic neuropathy have failed, in part due to the lack of a validated endpoint. The cornea has advantages that have densely innervated with various types of nerve fibers and these fibers are lost with experimental diabetes. These changes in nerve fiber density and tortuosity can be quantified non-invasively, rapidly, and serially by confocal microscopy using standard clinical instruments. Clinical observational studies show reduced corneal function prior to the onset of peripheral neuropathy and corneal confocal microscopy is more sensitive and predictive in detecting mild versus moderate peripheral neuropathy than skin biopsy or the clinical examination. Twenty-five patients treated with pancreas transplantations showed some increased fiber density 6 months of post-operatively suggesting nerve regeneration may follow resolution of diabetes. Work is ongoing to determine if corneal confocal microscopy could be a surrogate endpoint for clinical trials.

Ian Simpson and Rashmi Kumari (Penn State College of Medicine) discussed the increased risk and worse prognosis for stroke in persons with diabetes (7). Using the db/db model of type 2 diabetes, they find decreased expression of mRNAs for vascular endothelial growth factor (VEGF) and ciliary neurotrophic factor (CNTF), with increased interleukin-1 (IL-1) and tumor necrosis factor (TNF) alpha in animals

subjected to carotid artery occlusion followed by exposure to hypoxia. IL-1 expression colocalizes with microglial cells. There are also increased brain macrophages in the diabetic animals after 48 hours, and the microglia may be involved in macrophage recruitment. Female animals have less severe insults. Similar results are found in the ob/ob stroke model. The blood-brain barrier appears to be relatively intact. This work suggests that reduced and/or delayed expression of pro-survival factors such as VEGF, may contribute to the worse recovery after stroke in diabetic rodents and humans. That is, normal wound healing in which growth factors are involved is impaired in diabetes. This work is consistent with clinical findings described below by Dr. Biessels.

Tom Gardner and Ravi Singh (Penn State College of Medicine) emphasized the neurodegenerative components of diabetic retinopathy, including microglial cell activation, astrocyte and Müller cell dysfunction, and neuronal apoptosis (8). These changes occur shortly after the onset of experimental diabetes in rodents (1 to 3 months), prior to the development of overt vascular lesions such as microaneurysms or capillary occlusion (6 or more months). Therefore, diabetic retinopathy is a neurovascular disorder. The term “microvascular disease” does not describe the full spectrum of changes and its use should be reconsidered. Understanding these changes in animal models may guide future human testing to develop new functional endpoints for pre-clinical and mild background retinopathy, and may allow for earlier intervention in patients and better endpoints for clinical trials. They pointed out that regeneration occurs in the retina after detachment, macular hole repair, in some cases of macular degeneration treated with ciliary neurotrophic factor, and in diabetic retinopathy after reduction of elevated plasma lipids or blood pressure.

David Antonetti and Jeffrey Sundstrom (Penn State College of Medicine) discussed the mechanisms of VEGF-regulated retinal vascular permeability (9). The protein kinase C beta inhibitor, ruboxistaurin (Eli Lilly) reduces sustain visual loss from 9.1 to 5.5%, reduces the progression of diabetic macular edema and increases visual acuity. However, despite the clinical success of this drug, it has not received approval by the FDA, but for reasons that have not been made public. Their studies of regulation of paracellular vascular permeability focus on tight junction proteins such as occludin. VEGF and diabetes induce phosphorylation of occludin, a protein kinase C beta inhibitor reverses this effect, and partially inhibits endothelial cell permeability. Their work also reveals that tight junction proteins move from the plasma membrane into endosomes and are degraded, and suggest that protein kinase C zeta may also regulate vascular permeability. Further understanding of the role of these tight junction proteins should guide future drug therapy targets.

Kwok Fai So (Li Ka Shing Medical Facility University of Hong Kong) discussed the potential use of *Lycium barbarum* (Wolfberry) as an anti-apoptosis drug that may be useful for glaucoma, possibly by reducing the level of phospho-jun N-terminal kinase (JNK) 1 (10). Glaucoma is a very common retinal and optic nerve degeneration associated with increased intraocular pressure, and has its primary effects on retinal ganglion cells and their axons. *Lycium* extracts given orally to rats reduced ganglion cell loss without lowering intraocular pressure. This approach may yield means to provide neuroprotection to the retina and other parts of the CNS.

Session 3 examined lessons from development and stem cell applications for CNS regeneration in diabetes. Geert Jan Biessels and Ineka Brands (University of Utrecht) presented on the cerebral complications of diabetes (11). Diabetes mellitus is associated with changes in cognition. In patients with type 1 diabetes mellitus this is reflected in a mild to moderate slowing of mental speed and a diminished mental flexibility, whereas in type 2 diabetes impaired cognitive performance mainly involves learning and memory, mental flexibility and mental speed. Persons with diabetes have approximately 1.5-2 fold increased risk of Alzheimer’s disease and a 2 to 3 fold increased risk for “vascular” dementia. On brain MRI patients with type 1 diabetes have subtle reductions in brain volume. In patients with type 2 diabetes cortical and subcortical atrophy is relatively more evident than in type 1 diabetes, and is accompanied by vascular brain lesions. The clinical correlates of cognitive dysfunction and reductions in brain volume in type 1 diabetes are diabetes duration and the presence of other complications. In type 2 diabetes macrovascular disease and vascular risk factors may play an additional role. The role of insulin and insulin resistance was discussed. Insulin receptors are widely distributed in the brain and published reports suggest insulin plays an important role in learning and memory.

Animal models are available. Diabetic rats perform less well in a water maze test and have impaired memory function that improves with insulin treatment. There is also decreased cell proliferation in the dentate gyrus of diabetic rats. Taken together, these mechanistic and clinical studies strongly suggest that diabetes has a significant long term impact on brain function, particularly in type 2 diabetes, and may be considered as a new complication of diabetes.

Colin Barnstable and Joyce Tombrá-Tink (Yale University and Penn State College of Medicine) discussed neuroprotective and anti-angiogenic strategies for retinal diseases (12). Achieving homeostasis in the retina may be achieved through identifying signaling pathways where these processes intersect. They have employed retinal explants as a model to screen for means to restore lost retinal ganglion cells and have examined homeostatic factors that provide protective signals. These protective signal pathways that intersect but may have opposite effects in different cell types. For example, Stat 3 and Akt interact to regulate cell migration with a Müller cell migration. In particular, they have examined the role of the transcription factor, Stat3, which plays a major role in embryonic development and the differentiation of organ systems. It is also a key regulator of movement of proliferation and movement of retinal progenitor cells to a terminally differentiated complex. It is expressed in ganglion cells, Müller cells and retinal pigment epithelial cells. Stat 3 is up-regulated in retinal ischemia and promotes ganglion cell survival in the face of glutamate toxicity. Virally-introduced Stat3 can protect against glutamate toxicity in the retina and Stat3 conditional knockout mice gradually lose Müller glial cells. Another factor with important influences on the retina is pigment epithelial derived factor (PEDF). It increases differentiation, is anti-angiogenic, anti-tumor and neuroprotective against light, glutamate, hydrogen peroxide and pigment epithelial detachment. PEDF is expressed in the retina, and detected in aqueous and vitreous humor. A 10 amino acid peptide can be delivered by nanoparticles to protect the ischemic retinas and may be useful for longterm ocular therapy. It also inhibits neovascularization in the mouse retinopathy of prematurity (ROP) model. Together, these studies show that multiple factors influence retinal cell function and survival.

Maria Grant and Sergio Li Calzi (University of Florida) discussed human hematopoietic stem cell repair of vascular injury in the eye (13). Vascular nonperfusion and accelerated neovascularization are prominent features of diabetic retinopathy. Green fluorescent protein (GFP) chimeric mice allow tracking of transplanted cells to sites of vascular injury. Diabetes decreases the number of CD34-positive bone marrow derived endothelial cell progenitors in the blood and decreased proliferation into mature endothelial cells. A nitric oxide (NO) donor enhances migration and deformability of these cells. Endothelial cell progenitors migrate to injured capillaries after ischemic/reperfusion injury to stimulate re-endothelialization, and this process appears to be impaired in diabetes. Some of the defects in cellular motility are related to changes in vasodilator-stimulated phosphoprotein (VASP) interacting with actin, allowing actin to push against the plasma membrane. This interaction is stimulated by nitric oxide donor and its phosphorylation of VASP. Collectively, understanding these processes may lead to stem cell therapy for vascular dysfunction in the nervous system.

Patrizia Ferretti and Rachael Dobson (University College London) discussed neural stem cell recruitment for regeneration (14). They have used amphibian, chick and rodent retinas to understand the identity, plasticity and behavior of neural stem cell progenitors, and to provide trophic factors that may enhance their differentiation, integration, and function. Stem cell progenitors from young embryos have regenerative capacity superior to those from older embryos. Adult stem cells are located in the lateral walls of the lateral ventricles and hypoxia/ischemia increases neurogenesis in the subventricular zone, forming astrocytes and neurons in response to various trophic factors including VEGF, erythropoietin and stromal-derived factor-1 (SDF-1). Neurospheres can be labeled with iron oxides and detected with MRI. This method is FDA-approved for in vivo use. Understanding the various trophic factors that influence stem cell survival and function and having techniques to monitor their localization is important for development of this approach.

Session 4 examined potential and practical strategies to achieve regeneration in neural complications of diabetes.

Rutledge Ellis-Behnke and Gerald Schneider (MIT) presented work using peptide nanofiber scaffolds for nerve regeneration and a theoretical model for promoting CNS repair focusing on the four “p’s” in

regeneration: preserve, permit, promote and plasticity. Self-assembling peptides were used to develop a nanofiber meshwork used to promote peripheral nerve bridges in transected optic nerve in hamsters, evidenced by functional return of vision (15). The nanofiber scaffold creates a bridge that allows regenerating axons to grow through the site of injury. The density of connection is critical and this can be limited by scar formation or vascular injury. Dr Ellis-Behnke outlined a theoretical framework of steps required for neural regeneration which focused on creating a permissive environment for cell survival and growth, including blockade or removal of scars, and blockade of growth-inhibitory signals in tissue. Promotion of growth and myelination could be induced by exogenous factors, for example by local gene therapy. Thus, both a conceptual framework and nanotechnological approaches are important to understand how to develop useful CNS and peripheral nerve regenerative techniques.

Michael Young and Ji Yeon Kim (Schepens Eye Institute) discussed tissue engineering and transplantation of retinal stem cells. In specific conditions, brain-derived cells can develop into retinal neurons but this is hard to achieve. To replace photoreceptors in the post-mitotic retina, possible cell sources include embryonic or neural stem cells, or retinal stem cells. Retinal stem cells were isolated from mice, which self-renew in vitro on dissociation and when transplanted to sub-retinal space show a homing response and differentiation as evidenced by photoreceptor markers (16). The group is also using polymer scaffold approaches for seeding progenitor cells. Transplantation of cells into the subretinal space using these polymer scaffolds leads to a profound increase of transplanted cell survival and differentiation, as evidenced by expression of mature retinal markers not seen without the scaffold. However, thinner scaffolds are needed and consideration of biodegradability and biocompatibility will be required for this transplant technique to be optimized. In addition, slow-release polymer approaches to may improve the feasibility of local sustained growth factor delivery to the eye and other tissues, promoting cell survival and potentially regeneration. For example, release of glial-derived neurotrophic factor (GDNF) from poly (lactic-co-glycolic) acid (PLGA) microspheres promotes retinal ganglion cell survival in a mouse model of glaucoma and may be applied to other disease of the retina. This work shows that appropriate interactions of stem cells and their environment is key for their survival.

José-Alain Sahel and David Gaucher (St. Antoine Hospital, INSERM) discussed lessons from rod-cone photoreceptor dystrophies that might be applied to regenerative approaches to diabetic eye disease. Factors such as ciliary neurotrophic factor (CNTF) and GDNF protect against photoreceptor degeneration. Factors that impair retinal repair and regeneration include toxic by-products of rod cell degeneration, loss of structural support supporting cell changes, vascular changes, altered connectivity and trophic interdependence of photoreceptors. The group outlined an elegant approach to identify protective or survival factors from expression libraries transfected into Cos7 cells. Conditioned media from these cells was used in an assay to assess cone degeneration in chicken retina by automatic cell counting. Identification of diffusible factors from retina that preserve cone function identified a protein, Rod-derived Cone Viability Factor (RdCVF) a rod-derived protein important for neural cell survival (17). This factor, when injected intraocularly, shows a rescue effect that can be blocked by a RdCVF-specific antibody. David Gaucher discussed early neural complications in diabetic retinopathy. Diabetic retinopathy has been clinically defined as a microangiopathic disease. However functional alterations can be detected before visible vascular changes in the retina Microglial changes observed in rat models in the absence of neural apoptosis coincided with functional electroretinogram (ERG) changes indicating an early involvement of microglia in the disease process. This work suggests that protection and regeneration of neural cell types in retina are likely to be important approaches to the treatment or prevention of diabetic retinopathy.

Aviva Tolkovsky and Ed Bampton (University of Cambridge / University of Leicester) discussed work to dissect effects of hyperglycemia on components of the nervous system in mouse DRG and Schwann cell cultures. In the absence of Schwann cells, a loss of neurite length in DRG was observed under hyperglycemic conditions. Hyperglycemia may induce a proliferative block or defects in adhesion and migration of Schwann cells rather than apoptosis of these cells. Osteonectin, a factor secreted by Schwann cells, was identified in a conditioned-media screen to promote survival of retinal ganglion cells and process outgrowth (18). The supporting role of Schwann cells, including secreted neurotrophic factors, under hyperglycemic conditions will be a critical consideration in the development of regenerative approaches to diabetic peripheral neuropathy.

Session 5 dealt with biotechnology approaches to tissue regeneration and regulatory issues.

Three companies participated in this session: Stratatech (stratatechcorp.com), represented by Lynn Allen-Hoffman; ReNeuron plc (reneuron.com), represented by Eric Miljan and John Sinden; and Aegea Therapeutics (aegea.com), represented by Kim Hewitt.

Lynn Allen-Hoffmann and Christina Thomas-Virnig (Stratatech Corp) presented skin substitute derived from 2 cell types for the treatment of burns and chronic wounds. Plating of a foreskin derived cell line, NIKS (19), on dermal fibroblasts allows generation of a fully stratified epithelium with full barrier function. Both Drs. Allen-Hoffmann and Sinden discussed regulatory hurdles specific to cell therapy including the challenges and importance of developing scalable and GMP-compliant cell banks. John Sinden presented a case study for the clinical application of neural stem cells. The cell line ReN001 was derived from 12-week human fetal tissue and a subsequent cell line where growth is inducible by tamoxifen was created by insertion of a c-mycER transgene. This allows for scalability of manufacturing required for this to be a viable cell source for potential application in neural repair and regeneration. Preclinical data showed migration of these cells in a rat model into areas of stroke damage and improvement of sensorimotor function (20). The ensuing discussion emphasized the need to define, using appropriate markers, differentiated cell types of surviving transplanted stem cells. In addition, further insight into mechanism of action of cell therapies is required, for example whether beneficial effects are mediated by direct cell replacement, or trophic / support effects on sites of tissue damage. Kim Hewitt discussed targeting the Jnk pathway in peripheral neuropathy. Experimental diabetic peripheral neuropathy results in increase phosphorylation of mitogen-activated protein kinases (MAPK) including activation of Jnk (21). Aegea is developing neuroprotective compounds to inhibit the JNK pathway.

Summary and Recommendations

To the best of our knowledge the 11th EASD/JDRF Oxford workshop was the first assembly of experts on diabetic neurological disorders and neuro-regenerative medicine. Several general observations arose from the discussions:

1. Diabetes affects all mammalian tissues, with the most rapid metabolic impact in skeletal muscle, fat and liver. Recent work emphasizes that all parts of the nervous system undergo degenerative processes over the life of persons with diabetes. These changes range from subtle impairment of autonomic responses in the pupils, heart, intestines, skin and reproductive organs, to motor and sensory neuropathies, cranial nerve palsies, impaired cognition, retinopathy and stroke. These lesions cause tremendous patient suffering and current approaches beyond intensive metabolic control do not fully prevent the problems and provide little relief of disabling symptoms. From the perspective of patients and their families, much greater emphasis must be placed on prevention and early intervention because late-stage disease will remain difficult to recover.
2. The effects of diabetes on the motor and sensory nerves of different organs (brain, eye, feet, heart, intestinal and reproductive tracts) have been narrowly described by anatomical and functional differences with little understanding of potential common mechanisms. The clinical criteria for analyzing neuropathy are qualitative rather than quantitative and despite development of criteria for clinical research two decades ago, there are still no quantitative and validated end-points for clinical trials (22). Similar limitations also hold for other nervous complications.
3. Neurodegeneration develops due to primary metabolic insults to neurons, glial cells or vascular cells that impair normal homeostatic processes and loss of neurotrophins, as well as second hits, such as trauma, inflammation and infection. Strategies to stabilize or restore neuronal function may include restoration of metabolic and neurotrophic pathways and control of second hits. These approaches are summarized as: preserve, permit, promote and plasticity (Rutledge Ellis-Behnke). From a clinical standpoint this involves optimizing long-term and short-term stability of glycemic levels, control of blood pressure and lipid abnormalities, renal function, anemia, and chronic foot or dental infections.

A graphic approach to neurodegeneration and regeneration in diabetes is provided by Geert Jan Biessels (Figure 1). . Using stroke as a model, there is primary and secondary damage after the onset of stroke, and time-dependent recovery. Patients with diabetes have increased primary secondary damage and impaired long-term recovery. Similar relationships may also hold for other neurological complications of diabetes, including impaired cognition. These concepts may be useful for designs of the optimal timing for intervention in clinical therapeutic trials.

Figure 1: stroke in patients with diabetes mellitus

Diabetes mellitus is associated with an increased risk of stroke. Functional outcome after stroke is worse and post-stroke mortality is increased. Several factors may account for these effects. More severe pre-existent vascular pathology may increase the “primary damage” at the time of stroke, due to, for example, failing collateral flow. Immediately after stroke a cascade of events follows, including inflammation, oxidative stress and loss of ion and transmitter homeostasis, leading to “secondary cerebral damage”. Diabetes may affect several components of this cascade, thus increasing secondary damage. Finally, diabetes may hamper the recovery after stroke, due lack of compensatory mechanisms as a result of preexistent subclinical cerebral damage, and because of disturbances of endogenous repair mechanisms.

4. Regeneration of CNS tissues can be achieved by stem cell approaches in rodents but many questions remain about how to optimize the incorporation, viability and function of exogenous stem cells. Early stage clinical trials are now in progress for Parkinson’s disease and non-diabetic retinal degenerations.
5. Biotechnology firms that have developed from academic-based research laboratories are now acquiring the investment capital, intellectual property protection, and regulatory environments that may permit clinical translation of discovery science. Increased technology transfers will be required to realize the potential of fundamental research.
6. Animal models of diabetes have been very useful to study potential mechanisms of disease but only human investigations can provide the opportunity to validate clinical trial end-points that will be useful for disease classification, therapeutic testing and regulatory approval. Collaborative clinical studies will be needed to address these limitations.

Acknowledgements: The Oxford Workshops are hosted and organized jointly by EASD and the Juvenile Diabetes Research Foundation. The scientific Chairs for this 11th Workshop were Drs. Julia Polak and Tom Gardner. The Workshops’ scientific coordinator is Dr. Philippe Halban. EASD and JDRF thank Mrs. Mary Hata (EASD) for her wonderful job of organising this meeting and the sanofi-aventis company for their generous support.

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