Shedding new light on diabetic retinopathy with optical coherence tomography

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Citation for published version (APA):
Chapter 9

General discussion
Diabetic retinopathy

Diabetic retinopathy (DR) is one of the leading causes of blindness in developed countries. The clinically visible onset of DR with micro-aneurysms, capillary non-perfusion, hemorrhages and/or lipoprotein exudates has led to the assumption that DR has primarily a microvascular origin. The vascular focus is largely due to the fact that the retinal vascular abnormalities are visible by ophthalmoscopy, and that advanced vascular abnormalities correlate with and/or directly cause vision loss. The retina is a layered tissue lining the interior of the eye that enables the conversion of incoming light into a neural signal that is suitable for further processing in the visual cortex of the brain. The retina is an extension of the brain embryologically, and both can be considered to be neural tissue with vascular supply. Normal function of the retina depends on the interaction between neuronal cells, including photoreceptors, horizontal and bipolar cells, and amacrine and ganglion cells, and their supporting glia cells (astrocytes and Muller cells), as well as inner vascular (endothelial cells and pericytes) and outer blood-retinal (choroidal vessels and pigmented epithelium) barriers. The complexity and functional demands of the retina make it susceptible to eventual loss of tissue homeostasis in the presence of diabetes. Iadecola was the first to introduce the term neurovascular unit to describe the functional unit formed by neurons, astrocytes, smooth muscle cells and endothelial cells that controls cerebral blood flow in response to metabolic demand. Metea and Newman described this functional and structural interactions between neurons, glial cells, and vascular cells in the inner retina. Thus in both the retina and in the brain, neurovascular coupling regulates blood flow to meet the oxygen and nutrient demand created by electrical and metabolic activities. The retina is a neurovascular unit.

Functional retinal changes in diabetes

Retinal dysfunction in diabetes can be measured by a variety of psychophysical and electrophysiological methods, as well as by methods to measure blood flow changes. In people with diabetes who have no clinically visible signs of diabetic retinopathy the physiology of the neurovascular unit is altered. The ability of the retinal circulation to regulate blood flow in response to neural activity or metabolic demand is diminished. These alterations can be measured with flicker light stimulation of the retina. In the normal retina, the increase of neural activity from flicker light stimulation leads to an increase in blood flow and subsequent retinal arterial and venous dilation. In people with diabetes, even before the onset of structural microvascular abnormalities on dilated indirect fundoscopy and retinal color photographs, these flicker-induced blood flow changes are attenuated. The mechanism by which diabetes affects the neurovascular coupling response to flickering light is not clear.
Furthermore, several studies have shown using psychophysical and electrophysical methods that vascular changes in the retina of diabetic patients are accompanied or even preceded by neurodegenerative changes.9-16

**Psychophysical methods**

Visual acuity is the most commonly used functional test of the retina. However, in the early stages of diabetic retinopathy visual acuity is normal, because this test measures the foveal function which is not impaired until the later stages of diabetic retinopathy. Standard automated perimetry, microperimetry, frequency doubling technology, short wavelength automated perimetry and Rarebit technique all show a reduction of retinal sensitivity outside the fovea in diabetic patients even in the absence of DR.17-22 Contrast sensitivity is also diminished in the absence of vasculopathy.23 Color vision defects such as tritan color confusion and loss of sensitivity to blue light are demonstrated to occur in diabetic patients.24, 25

**Electrophysiological methods**

Electrophysiological methods allow the evaluation of the entire visual pathway. The function from retinal photoreceptors to the occipital primary visual cortex, can be objectively assessed by recording cortical potentials evoked by patterned stimuli (visual evoked potentials). There are several studies showing VEP responses with an increased implicit time and a decreased amplitude in persons with type 1 diabetes with and even without DR.26-28 Abnormal VEPs observed in people with diabetes indicate a general involvement of the visual system or of one of its composing structures. Furthermore, in healthy control subjects, photostress induces VEP changes consisting of an increase in implicit time and decrease in amplitude, which recovers in a range of between 68 and 78 seconds.29 However in people with diabetes with and without vasculopathy this implicit time after photostress is increased, the amplitude is lower and the recovery time is increased compared to control subjects.30 This abnormal VEP results after photostress suggest macular dysfunction in the diabetic patient.

The electroretinogram (ERG) is an electrophysiological test that provides objective and quantitative information on retinal (dys)function by recording electoretinographic signals evoked by a light stimulus (flash or pattern). A full field response is generated by stimulating the entire retina with a flash light stimulus. Major components of the electrical waveform thus measured include the a-wave, primarily derived from the photoreceptors; the b-wave, derived from the inner retina (probably Muller and bipolar cells); and the c-wave, derived from the RPE and photoreceptors. The oscillatory potentials (OP) are low-amplitude, high-frequency wavelets superimposed on the ascending limb of the ERG b-wave. The exact intraretinal sites from which the OPs originate are unknown.31 Retinal function as measured by OPs can be abnormal in diabetics before vascular changes are clinically observable.28, 32-34 Bresnick et al. showed that the reduction in OP amplitudes
predicts progression of DR. The ERG response generated by a pattern stimulus, is thought to reflect the ganglion cell activity. Impaired pattern ERG responses are present in diabetes patients with disease duration ranging from less than 6 months. Multi-focal ERG (mfERG) is a technique used to measure the function of localized regions of the retina. With mfERG it is therefore possible to detect focal retinal abnormalities. Studies using mfERG revealed that measuring focal areas of increased implicit time can predict the development of retinal vascular changes in patients with diabetes.

**Structural retinal changes in diabetes**

The microvascular changes visible with ophthalmoscopy are microaneurysms, hemorrhages, lipid exudates, macular edema, capillary occlusion, cotton-wool spots and neovascularizations. Chronic exposure to hyperglycaemia is hypothesized to initiate a cascade of biochemical and physiological changes that ultimately lead to microvascular damage. Retinal pericyte loss from the retinal capillaries is a characteristic early feature of preclinical DR. Normal pericytes have a contractile function involved in blood flow autoregulation in the retina. The loss of pericytes is accompanied by the loss of capillary endothelial cells and presumably vasoregression, the loss of capillaries. In response to the subsequent hypoxia, retinal cells express VEGF and other stress factors, stimulating increased capillary permeability, microaneurysm formation and parainflammation. Affected areas tend to enlarge, possibly by a vicious circle of VEGF-induced vascular closure and ischaemia, resulting in focal or diffuse retinal vascular leakage threatening the function of the macula, or in worse cases in pre-retinal vascularization causing blindness by hemorrhage or scar formation. Diabetes not only causes microvascular changes. The above mentioned functional studies indicate that there is an early retinal neurodegenerative component in people with diabetes. This early retinal neuropathy observed in diabetic patients is confirmed by studies showing alterations in structure of different retinal cell types. Histopathologic studies emphasized the loss of neurons in human diabetic retinopathy more than 40 years ago. Barber et al. showed that experimental streptozotocin (STZ) diabetes in rats and diabetes mellitus in humans are accompanied by increased apoptosis of retinal neural cells. This occurred already after only 1 month of experimental diabetes in rats, and a similar increase in apoptosis was noted in a human retina after six years of diabetes. The STZ diabetic rats had significantly reduced inner retinal layer thickness 7.5 months after onset of hyperglycemia, suggesting that the diabetes induced apoptosis leads to loss of cells in the inner retinal retina. Apoptosis was also increased in the Ins2Akita mouse model of type 2 diabetes to a similar extent as in STZ diabetic rats. Data from human retinas suggest that there are similar increases in apoptosis, which indicates that these animal models accurately reflect the apoptosis in the human retina in diabetes. The inner retinal layer thickness was reduced in Ins2Akita mice after 5 months of hyperglycemia. Several studies have demonstrated loss of ganglion cells in animal models of diabetes. Barber et al made an approximation of 10% reduction of ganglion cells after 7 months of diabetes.
Another study with retinas of STZ diabetic mice identified a 20-25% reduction of ganglion cells after 14 weeks of hyperglycemia. Besides accelerated apoptosis of retinal neurons, glial activation, impaired glial cell metabolism, have also been found in animal studies. Several studies have measured the thickness of the retinal nerve fiber layer (RNFL). The thickness of the RNFL is assumed to be related to the number of ganglion cells. Chihara et al. found defects in the thickness of the RNFL using red free fundus photography in 20% of the patients without vascular retinal changes such as microaneurysms. With the use of scanning laser polarimetry several studies have found a correlation between RNFL layer thinning, glycemic control, duration of DM and degree of DR.

Optical coherence tomography

With optical coherence tomography (OCT) and advanced segmentation algorithms, it has become possible to image the human retina longitudinally and quantitatively in vivo and to measure total retinal thickness (RT) and the thickness of specific layers with high accuracy. Several groups have shown that total RT is decreased in patients with no or minimal DR compared with healthy controls. As the retinal layers are affected differentially by diabetes it is desirable to quantify thickness of the separate layers within the retina. Automated three-dimensional segmentation algorithms (such as the Iowa Reference Algorithms http://www.iibi.uiowa.edu/content/downloads) allow the measurement of the thickness of the individual retinal layers in time domain OCT (TD-OCT), spectral domain and swept source OCT (SD-OCT) scans. The studies described in this thesis show that the thinning of the previously described pericentral macula from TD-OCT images in patients with diabetes without DR is due to a selective loss of thickness in the inner retinal layers, in particular the ganglion cell layer (GCL) plus inner plexiform layer (IPL). However, the individual layers of the retina that can be distinguished on SD- OCT images allow for an even more detailed analysis of the individual layers. Measurements of individual retinal layers thickness with segmentation of SD-OCT images demonstrated GCL thinning in the pericentral area and corresponding loss of RNFL thickness in the peripheral macula in patients with type 1 diabetes with minimal DR compared with control subjects. Additionally, in type 2 diabetes patients with minimal DR the inner retinal layers in the macula are thinner than in controls. Other research groups also measured inner retinal layers thickness changes in the macula of people with diabetes without minimal or no DR, and their results confirm the diminished inner retinal layer thickness in diabetes compared to control subjects as described in this thesis. These RNFL and GCL losses, though significant in groups of patients, are not large enough to characterize individual patients. The reason is that single measurements show high variability between individuals, both normal and diseased, and standard single cut-off values, are too insensitive to detect differences changes. Longitudinal observations detecting significant trends of change could be more useful in this respect. Recently it was demonstrated, that the thinning of the inner retinal layers – specifically RNFL, GCL, and IPL - in the macula is
progressive over time in patients with type 1 DM, with no or minimal vasculopathy and that duration of diabetes appears to be a key determinant. Interestingly, neither the presence of vasculopathy, nor an increase of vasculopathy during the observation period, was significantly related to this decrease in layer thickness. With several studies demonstrating decline of the neuroretina in vivo in humans, it has been proven to be possible to measure and quantify, both microvascular damage, and neural damage in the retina.

**Is there a relationship between vascular diabetic retinopathy and diabetic retinal neuropathy?**

The molecular mechanisms involved in retinal neurodegeneration in diabetes are complex and may include a combination of ocular factors such as increased oxidative stress, loss of neuroprotective factors, increased inflammation, glutamate excitotoxicity, and systemic factors including hyperglycemia, dyslipidemia, and insulin deficiency. The exact relationship, if there is any at all, between vascular diabetic retinopathy and diabetic retinal neuropathy is not yet known. Diabetic retinal neuropathy could be a secondary result of vascular damage, itself the result of hyperglycemia, leading to increased permeability and occlusion of the retinal microvasculature and subsequent neuronal loss. In this scenario diabetic vasculopathy would probably be observed preceding the retinal neuropathy. An alternative hypothesis is that diabetes primarily affects the neuroretina, and that this secondarily compromises vascular integrity by an unknown mechanism, in which case diabetic vasculopathy is preceded by diabetic retinal neuropathy. It is also possible that these pathological changes occur independently, as two separate sequelae of the diabetic state.

The studies mentioned previously, demonstrating structural or functional retinal neuropathy, were performed in diabetic patient or diabetes animal models with no or minimal DR. Thus, they indicate neuroretinal degeneration to be one of the earliest detectable retinal abnormalities in patients with DM, and possibly even preceding vasculopathy. Most of the functional studies in diabetic patients without apparent DR do not exclude the possibility of pre-clinical vascular changes, not visible with funduscopy or fundus photography. Such subtle capillary dropout or changes in the blood-retinal barrier due to diabetes can be visualised on fluorescein angiogram (FA) and vitreous fluorometry (VF). Interestingly, Reis et al. recently found evidence for neuropathy in the absence of vascular DR in patients with diabetes type 1. They described implicit time changes measured with mfERG and impaired contrast sensitivity in the absence of breakdown of the blood retina barrier or capillary dropout, as demonstrated with FA and VF. Yoshida et al. performed electroretinography and vitreous fluorophotometry in diabetic patients and healthy control subjects. They found a delay in the peak implicit time of the oscillatory potential in the people with diabetes and no significant difference in the permeability of the blood-retinal barrier between the two groups. These results emphasize that the retinal functional changes may be present before changes in blood-retinal barrier permeability are apparent. Harrison et al. recently showed that with the use of mfERG the onset of diabetic retino-
pathy can be predicted within small retinal patches. Di Leo measured the natural course of diabetic retinal dysfunction in a group of diabetic patients and no DR with fluorescein angiography and focal electroretinogram at the macula, for 3 years. The results suggests that in diabetic patients the neuronal function of the retina was surprisingly impaired after only a few years of disease before clinically detectable vascular abnormalities occurred. Two other longitudinal studies demonstrating progression of neurodegeneration both show no association between progressive neurodegeneration and the presence or progression of vascular diabetic retinopathy. Hellgren et al detected progression of early retinal dysfunction with standard automated perimetry (SAP) in diabetic patients and no or minimal DR. Visual field deterioration was not correlated with a change in retinopathy. Van Dijk et al. showed that the thinning of inner retinal layers in the macula in diabetes type 1 is progressive over time and is related to disease duration but occurs independently of retinal vasculopathy.

However, contradicting this, a recent study performed by Lasta et al. indicates that the retinal vascular response to flicker stimulation is reduced before the reduction in pattern ERG in patients with type 1 diabetes without DR. This seems to indicate that in early diabetes, the abnormal retinal response may not be a consequence of reduced neuronal activity. However, they used pattern ERG instead of mfERG, and the latter is superior in measuring early retinal neurodegeneration in diabetic patients.

Though the results of the above studies as well as our results may sometimes contradict each other, in general they suggest that both processes are independent of each other. However, both processes once established, dependent or independent of each other, will probably negatively influence one another, because the retina behaves as a neurovascular unit in which neurons, glial and microglial cells, and blood vessels are organized to facilitate adaptations to varying conditions.

Neuroprotection

The standard of care of newly diagnosed DR relies on laser photocoagulation and injection of anti-VEGF agents. However, laser treatment is not uniformly successful in halting visual decline and is associated with side effects such as moderate visual loss, diminished visual field, reduced color vision, and reduced contrast sensitivity. Although success has not been total, the anti-VEGF treatments represent a major advance in DR treatment. The anti-VEGF treatments demonstrate that treating the vascular aspects in the later stages of the disease can preserve vision in many patients. However the greater goal is to prevent the onset of the disease or arrest its progression at a stage preceding the appearances of microvascular pathologies so that patients with diabetes can maintain vision without the need for invasive or destructive procedures such as intravitreal injections with anti-VEGF and photocoagulation. The local availability of growth factors and neurotrophins, which are peptides that promote neuronal differentiation and survival, are essential for the survival of retinal neurons in a hostile environment. In diabetes, the efficacy and/or
The concentration of such molecules are reduced, suggesting that treatment with neuroprotective factors has the potential to play a role in preventing vision loss associated with diabetes. The debate concerning the exact relationship between the vascular diabetic retinopathy and diabetic retinal neuropathy is relevant when we think in terms of possible neuroprotection in the future. When neurodegeneration precedes and possibly causes or accelerates the microvascular changes in DR, then retinal neuroprotection could possibly prevent or inhibit these microvascular changes and prevent vision loss. Even when both processes are independent, it is important to realize that due to retinal diabetic neuropathy the diabetic patient suffers vision loss such as color vision changes, diminished retinal sensitivity, and contrast sensitivity. The possibility of topical therapy delivering neuroprotective agents could open up a new and safe strategy for the prevention or treatment of the early stages of DR and diabetic neurodegeneration.

**Biomarkers**

Functional and structural measurements of retinal neurodegeneration and neurovascularopathy in DM patients are excellent candidates to serve as biomarkers for early retinal damage, and could be used in future trials exploring interventions to prevent the late vascular, visual acuity threatening complications of diabetic retinopathy. Moreover, these multiple biomarkers will possibly allow improved and early identification of persons with diabetes at increased risk for complications not only in the retina but possibly also in other organs.

There is increasing evidence that longstanding diabetes is associated with cognitive decline, cerebral atrophy, white matter hyperintensities and infarctions. In one study, these changes seem not to be related to microvascular complications of diabetes, and were found not be associated with retinopathy. Van Duinkerken et al found that compared with healthy control subjects, functional connectivity and cognition differed in type 1 diabetic patients irrespective of microvascular complication status, indicating that chronic hyperglycemia, among other factors, may negatively affect brain functioning even before microvascular damage becomes manifest. As the retina is an extension of the brain and the high embryonic and cellular similarity of neuroretinal tissue and neocortical tissue, it is reasonable to expect to find similar pathologic changes in both. Easily imaged and quantified retinal biomarkers could possibly be used as biomarkers for early brain damage due to diabetes.
REFERENCES


