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Neurodegeneration in diabetic retinopathy: Potential for novel therapies

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ABSTRACT

The complex pathology of diabetic retinopathy (DR) affects both vascular and neural tissue. The characteristics of neurodegeneration are well-described in animal models but have more recently been confirmed in the clinical setting, mostly by using non-invasive imaging approaches such as spectral domain optical coherence tomography (SD-OCT). The most frequent observations report loss of tissue in the nerve fiber layer and inner plexiform layer, confirming earlier findings from animal models. In several cases the reduction in inner retinal layers is reported in patients with little evidence of vascular lesions or macular edema, suggesting that degenerative loss of neural tissue in the inner retina can occur after relatively short durations of diabetes. Animal studies also suggest that neurodegeneration leading to retinal thinning is not limited to cell death and tissue loss but also includes changes in neuronal morphology, reduced synaptic protein expression and alterations in neurotransmission, including changes in expression of neurotransmitter receptors as well as neurotransmitter release, reuptake and metabolism. The concept of neurodegeneration as an early component of DR introduces the possibility to explore alternative therapies to prevent the onset of vision loss, including neuroprotective therapies and drugs targeting individual neurotransmitter systems, as well as more general neuroprotective approaches to preserve the integrity of the neural retina. In this review we consider some of the evidence for progressive retinal neurodegeneration in diabetes, and explore potential neuroprotective therapies.

1. Introduction

DR is the leading cause of vision loss in working-age adults, affecting more than 7.5 million people in the USA and approximately 190 million worldwide (Lee, Wong, & Sabanayagam, 2015). The prevalence of DR continues to increase due to the expansion of our ageing population and increasing prevalence of diabetes (Lee, Feldman, Ostermann, Brown, & Sloan, 2003). The clinical diagnosis of DR relies primarily on the detection of vascular lesions by ophthalmic fundus exam. The presence and distribution of microaneurysms, intraretinal hemorrhage, venous beading and neovascularization are used to define the stage of disease. DR commonly involves macular edema due to compromise of the blood retinal barrier, but the disease can progress in some cases to the proliferative form involving neovascularization. The presence and abundance of macular edema is now regularly detected by using spectral domain optical coherence tomography (SD-OCT). Using this technology, evidence from animal models of diabetes as well as clinical studies suggests that the neural retina is also affected by diabetes in ways that may have direct impact on visual perception.

In the last 15–20 years it has become apparent that the neural retina suffers from irreversible loss of neurons and degenerates in a manner analogous to that seen in other chronic neurodegenerative diseases (Barber, 2003). This process was initially characterized by accelerated loss of neurons by apoptosis, especially within the inner retina (Barber et al., 1998). The retinas of both animal models and humans with diabetes also exhibit other features of neurodegenerative disease including structural and biochemical changes that can potentially alter the function of individual neurons and even result in changes to the way the neural network processes information and delivers signals to the brain (Barber, Gardner, & Abcouwer, 2011).

Current treatment of DR includes laser surgery and, more recently, intraocular injection of anti-VEGF agents have been used for both diabetic macular edema and proliferative retinopathy (Wells et al., 2015). Anti-VEGF therapy has showed good success in treating macular edema, and is as equally effective as pan-retinal photocoagulation laser surgery in treating the proliferative disease (Network et al., 2015). These approaches have provided increased success in stabilizing the vascular pathologies of the disease and have led to improved visual outcomes. Disease severity, as graded and staged by vascular lesions, has also been shown to regress, but it is unclear whether other changes

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within the retinal tissue are reversible using this approach. Laser surgery undoubtedly causes permanent damage to the retina and has been shown to induce thinning of the retinal nerve fiber layer (Lim et al., 2009). Trials are ongoing to treat diabetic patients with anti-VEGF agents to prevent the development of DR as defined by vascular lesions, however, there has been little consideration of potential therapies targeting neurodegeneration. For the purpose of this review we will consider neurodegeneration, defined as a pathological condition effecting the structure or function of neurons, including their accelerated cell death, as discussed by Przedborski, Vila, and Jackson-Lewis (2003).

The relationship between vascular pathology and retinal neurodegeneration is still debatable. Whether or not these two aspects of the disease are causally related or independent phenomena is not established, and direct causality should not be assumed (Barber, VanGuilder, & Gastinger, 2007; Barber et al., 2011). It makes sense to continue to identify novel therapeutic targets that can be used to prevent the onset of DR, including the different features of neurodegeneration. Novel therapies for DR could include neuroprotective agents, as well as drugs to correct abnormalities in neurotransmitter release, uptake and neurotransmitter receptor signaling. Here we attempt to consider potential therapies for DR that target neural systems and neurodegeneration.

2. Diabetic retinopathy as a neurodegenerative disease: Retinal apoptosis

The most widely recognized feature of neurodegenerative disease is the accelerated loss of neurons by apoptosis. Increased frequency of apoptosis is now considered an important component of the pathology in DR. Early studies characterized the vascular lesions in postmortem specimens of human retinas (Bloodworth, 1963; Bloodworth & Molitor, 1965; Cunha-Vaz, 1978), but some of these also identified features of degeneration a decade before the concept of apoptosis (previously called pyknosis) was even characterized as a form of cell death with well-defined biochemical features (Bloodworth, 1962; Wolter, 1961). Later work used animal models of diabetes to identify and count individual apoptotic cells using terminal dUTP nick end labeling (TUNEL) in the vascular network isolated by trypsin digest (Mizutani, Kern, & Lorenzi, 1996). When this approach was adapted to the entire retina as a flat-mount, a much greater number of TUNEL-positive apoptotic cells could be observed and counted, suggesting that the cell death was not limited to just the vasculature but included cells within the neural retina, such as neurons and possibly glia (Barber et al., 1998). The increase in apoptosis was significant even in rats after only one month of streptozotocin (STZ)-diabetes, and continuing at a fairly constant rate over a 12-month period, suggested the possibility of chronic neurodegeneration. Other studies using TUNEL confirmed the increase in apoptosis in retinas of diabetic rats and mice (Martin, Roon, Van Ells, Ganapathy, & Smith, 2004; Barber et al., 2005), and in the KKAY mouse, which is thought to model Type II diabetes (Ning et al., 2004). Chronically elevated intraocular pressure was found to further increase the number of diabetes-induced TUNEL-positive cells, suggesting that a combination of glaucoma and diabetes could be even more damaging to the neurons of the retina (Kanamori, Nakamura, Mukuno, Maeda, & Negi, 2004). Since those early studies, apoptosis has been used as an endpoint in numerous animal studies modeling DR (Barber et al., 2011). Retinal apoptosis has also been noted in several postmortem studies of human retina, thus confirming the results from animal studies (Kerrigan, Zack, Quigley, Smith, & Pease, 1997; Abu-El-Asrar, Dralands, Missotten, Al-Jadaan, & Geboes, 2004a; Barber et al., 1998).

Other markers have been used to confirm retinal apoptosis in animal models of diabetes. The presence of cleaved caspase-3 in retinas of diabetic animals confirmed and extended the initial observations of cell death in the vasculature and neural retina of diabetic animals (Kowluru & Koppolu, 2002; Mohr, Xi, Tang, & Kern, 2002; Martin et al., 2004; Sasaki et al., 2010), including a rat model of Type II diabetes (Yang et al., 2013). A histological study noted that cleaved caspase-3 co-localized with cell-specific markers of neurons in whole-mount retinas from $Ins2^{Akita}$ diabetic mice (Gastinger, Singh, & Barber, 2006), and caspase-3 activity in diabetic rodent retinas was also measured biochemically (Brucklacher et al., 2008). Other immunohistochemical studies have revealed that pro-apoptotic BAX, Fas and caspase-3 were upregulated in the retinal ganglion cells of diabetic donors (Podestà et al., 2000; Abu-El-Asrar, Dralands, Missotten, Al-Jadaan, & Geboes, 2004b), whereas the anti-apoptotic BCL2 colocalized with GFAP positive cells, indicating its upregulation in Muller cells (Abu-El-Asrar et al., 2004b). The enzyme activities of caspase-3, caspase 12, Bad and BCL2 have also been shown to increase in diabetic rodent retinas (Brucklacher et al., 2008; Tang et al., 2011; Dong, Jin, Lu, & Kang, 2013).

While biochemical approaches and TUNEL labeling lack cell-type specificity several histological approaches have been used to identify the cells most affected by apoptosis. Gastinger counted choline acetyltransferase- and tyrosine hydroxylase-positive cells in flat-mounted retinas to establish that the number of both cholinergic and dopaminergic amacrine cells is depleted in the $Ins2^{Akita}$ diabetic mouse (Gastinger et al., 2006), and further showed that caspase-3-positive cells were located in multiple layers of the retina including occasional cells in the outer nuclear layer, suggesting that a small number of photoreceptors may also undergo apoptosis. Another study in STZ-diabetic rats suggested that a much larger number of photoreceptors die even after a short duration of diabetes (Park et al., 2003) although this result has not been replicated in other histological studies. When CFP was placed under the Thy-1 promotor in $Ins2^{Akita}$ mice there was a smaller number of surviving CFP-positive cells, confirming the specific loss of retinal ganglion cells in this model (Gastinger, Kunselman, Conboy, Bronson, & Barber, 2008).

One important aspect to consider is the impact of glycemic control on diabetic complications. Animal models are often studied in the absence of exogenous insulin. A study in diabetic dogs with moderate glycemic control using insulin ameliorated the diabetes-induced loss of optic nerve axons (Howell, Mekhail, Azem, Ward, & Kern, 2013). Similar results were found previously in rats treated with insulin (Barber et al., 1998), suggesting that the survival of retinal ganglion cells is determined by the severity of diabetes.

If apoptosis of neurons continues over an extended period of time there will eventually be a significant reduction in neural tissue volume. Tissue loss in the retina, being a highly topographically organized tissue, can be measured relatively easily using histological approaches. Many studies of diabetic animal models have revealed loss of the thickness of cell layers in the retina, especially the inner retina. The chronic loss of irreplaceable neurons was suggested by a reduction in the thickness of the inner plexiform and inner nuclear layers, as well as fewer large-bodied cells in the ganglion cell layer of retinas from rats that were diabetic for 7.5 months (Barber et al., 1998). Similar results were found by histological measurement of the retinas of $Ins2^{Akita}$ diabetic mice (Barber et al., 2005; Smith et al., 2008). Although less frequent, some studies have also reported loss of outer nuclear layer cells and consequent reductions in retinal thickness and there may be other degenerative changes affecting photoreceptors that precede cell death (Zhang et al., 2008; Enzsoly et al., 2014). For the most part there appears to be a consensus that a protracted period of diabetes leads to reduction in retinal thickness in a variety of animal models (Clermont et al., 2011; Yang et al., 2013).

3. Evidence of neurodegeneration from clinical imaging studies confirms the histological results from animal models of diabetes

The significance of apoptosis and progressive loss of cells in the retina in animal models of diabetes was difficult to determine. Apoptosis is a transient event so the number of TUNEL-positive cells