1. Neurodegenerative metabolites and neuroprotective strategies in diabetic retinopathy

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Abstract. Diabetic retinopathy is widely considered a neurodegenerative disease. A number of cellular and molecular studies showed that retinal neuronal cells are vulnerable to be damaged soon after diabetes. In addition, retinal function tests also confirmed functional deficit in diabetic retina early in diabetes indicating dysfunction of neuronal cells. Altered levels of diabetes induced neurodegenerative metabolites have been found to be implicated in initiating those damages. Those metabolites include increased levels of glucose, glutamate, branched chain amino acids and homocysteine and decreased levels of certain vitamins such as folic acid and vitamin-B12. These altered metabolites activate a number of metabolic pathways leading to increases in oxidative stress and decreases in neurotrophic support in the diabetic retina. In turn, they damage neurons in diabetic retina. In this chapter, we discuss those potential excitotoxic metabolites and possible therapeutic targets to protect neurons. By protecting retinal neuronal damage early in diabetic retina, retinal vessels can be regressed to ameliorate the progression of diabetic retinopathy, a leading cause of blindness worldwide.

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1. Introduction

Diabetic retinopathy (DR) is the leading cause of blindness among working adults worldwide. Earlier, DR was considered solely a vascular disease; therefore, most of the treatments options developed were based on targeting retinal vasculature at late stages of the disease. However, it is now widely recognized as neurodegenerative disease. A number of retinal function tests in diabetic patients, as well as cellular and molecular studies in the retinas of diabetic animals, suggest that neurons are vulnerable to be damaged shortly after the onset of diabetes [1,2]. In addition, several clinical tests indicate functional deficits in the neuronal component of retinas during the early stages of diabetes [3,4]. We and others have reported that diabetes causes a chronic loss of retinal neurons by increasing the frequency of apoptosis and the activation of glial cells [1, 2, 5-8]. Both retinal glia and neurons are compromised early in the disease progression and display altered metabolic functions and deregulated neurotrophic support.

It has been shown that retinal neurodegeneration causes early microvascular changes that occur in DR. These changes include the breakdown of the blood-retinal barrier (BRB), vasoregression and impairment of neurovascular interactions [5,9-13]. Transgenic mice model suggest that neurodegeneration and glial activation initiate vasoregression [12]. In addition, Kusari and coworkers reported that both memantine and bromidine treatments in diabetic animals exhibited neuroprotection in addition to reduced VEGF protein levels and reduced blood retinal barrier breakdown [10,11]. Therefore, neurodegeneration is believed to be upstream regulator for vascular damage in DR. Strategies to protect neurons early in diabetic retina may regress microvascular damage later in DR.

Diabetes-induced metabolic stress is considered the major initiating factor to cause damages to those neuronal cells in the retina. During the past few decades, hyperglycemia has been considered the main contributor and upstream inducer in the progression of DR. However, a number of studies suggest that excess glucose may not account for the range of cellular and functional changes in the progression of DR [14–16]. In addition to high glucose, the dysregulated levels of excitotoxic metabolites, such as glutamate, homocysteine, branched chain amino acids, nutrients, hormones and several other factors, all have been found to be implicated in neurodegeneration early in DR [17,18]. Therefore, better understanding of the neurogenerative metabolites, pathways and new therapeutic targets are needed to protect neurons early in DR. In this chapter, we discuss recent advances in understanding the neurogenerative metabolites, metabolic
stresses and neurotrophic factors in diabetic retina, as well as possible neuroprotective strategies.

2. Neurotoxic metabolites and neuroprotection strategies:

1. Hyperglycemia induced-oxidative stress and neuroprotection

Numerous studies have shown that hyperglycemia/diabetes activates several metabolic pathways. These pathways mediate oxidative stress, amplifying retinal tissue damage, which plays an important role in the pathogenesis of diabetic complications, including DR [17,19]. Sources of oxidative stress may include hyperglycemia-induced increased flux through the polyol pathway, depleting NADPH and lowering intracellular levels of the endogenous antioxidant glutathione. This leads to the activation of nuclear factor kappa B (NF-κB), increasing proinflammatory cytokines, chemokines and growth factors, causing further damage to tissue. Other hyperglycemia-induced pathways include activation of protein kinase C and increased flux through advanced glycation end products and hexosamine pathways, resulting in increased oxidative stress. Hyperglycemia-induced NADPH oxidase activation, microglial activations and NADH oxidases have also been found to increase oxidative stress in the diabetic retina. Oxidative stress has a neurodegenerative influence in the diabetic retina which has been shown to be prevented by constant intake of antioxidant [20]. Feeding rats with several anti-oxidants, including alpha-tocopherol, N-acetyl cysteine, ascorbic acid and beta-carotene, inhibited the increase in apoptosis. A recent study found that a polyphenol, epigallocatechingallate protected the retina against glutamate toxicity via an antioxidant mechanism [21]. Thus antioxidant inhibition offers important opportunities for future neuroprotective treatment against neuronal cell death in DR. Pigment epithelial derived factor (PEDF) has been shown to block pathways that lead to the production of ROS and found to have neuroprotective effect [64, 22]. Therefore, anti-oxidant therapy may be useful as an adjuvant treatment in combination with other treatments for the prevention and/or treatment of neuronal damage early in diabetic retina.

2. Strategies to inhibit glutamate excitotoxicity

Numerous studies indicate that due to the disruption of glutamate homeostasis in the diabetic retina, toxic levels of extracellular glutamate in the retina increases, which damage the neurons and initiate the development of DR [23–25]. The major cause of neuronal cell death following glutamate-
induced activation of \( N \)-methyl \( D \)-aspartate (NMDA) receptors is the influx of calcium and sodium into the cells. This generates free radicals and induces apoptosis [26]. Therefore, strategies to decrease the level of extracellular glutamate or to inhibit the activation of NMDA receptors may decrease neurotoxicity and cell death [25].

Müller cells in the retina are specialized cells, which regulate the level of glutamate both intra- and extra-cellularly within the retina. Müller cells maintain glutamate homeostasis. They immediately take up excess extracellular glutamate released during neurotransmission, thus lowering the excitotoxic level of glutamate. Within Müller cells, glutamate gets converted to either glutamine or to \( \alpha \)-ketoglutarate. The \( \alpha \)-ketoglutarate formed then enters into the citric acid cycle. Producing glutamate from the citric acid cycle intermediates requires a nitrogen source. This is provided by excess branched chain amino acids (BCAA), using either of the two isoforms of branched-chain aminotransferase (BCAT): the mitochondrial branched-chain aminotransferase (BCATm) expressed in Müller cells or the cytosolic BCAT isoform (BCATc), which is expressed only in the cytosols of neuronal cells [27,28]. We showed that in the retina, BCATm is expressed exclusively in Müller cell mitochondria, whereas BCATc is expressed in the cytosols of retinal neurons [29]. Gabapentin is a very specific BCATc inhibitor that is approximately 100-fold less active for BCATm. Our experiments showed that gabapentin inhibited glutamate synthesis from \( CO_2 \) and pyruvate [30,31]. The addition of BCAAs to the medium surrounding the excised retinas increased the oxidation of glutamate to pyruvate, lactate and \( CO_2 \) [27,30]. Therefore, the increased ratios of branched-chain keto acid (BCKA) to BCAA will decrease Müller cell glutamate synthesis and will increase rates of glutamate oxidation [25], whereas decreasing the BCKA/BCAA ratios in diabetic retinas will promote glutamate excitotoxicity by raising the levels of glutamate in Müller cells. Thus high serum BCAA level in diabetic state may be responsible for neuronal damage, because it may lead to high intra- and extra-cellular glutamate levels in the retina [25]. Thus, the biochemical mechanism of glutamate excitotoxicity suggests an increase in cell death, resulting in a loss of visual function in diabetes.

The intraocular administration of MK-801, an \( N \)-methyl \( D \)-aspartate receptor antagonist, has been shown to protect against neurodegenerative conditions [32]. Memantine, another NMDA receptor antagonist, demonstrated a neuroprotective effect in retinal ganglion cells (RGCs) when exposed to glutamate [33]. Memantine treatment in animal models of diabetes showed neuroprotection in addition to reduced vitreoretinal VEGF protein levels and reduced blood retinal barrier breakdown [10]. Recently, Smith and coworkers showed that a specific sigma receptor-1 ligand,
pentazocine, conferred significant neuroprotection in an in vivo model of retinal degeneration. Thus, sigma ligand may be a potential therapy for neurodegenerative diseases of the retina [34]. In a recent study, we found that memantine and gabapentin (Neuritin) administration to diabetic rats reduced caspase-3 activity and reduced the increased levels of ROS in the diabetic retina, suggesting these agents may protect neuronal cells (unpublished data). Thus, strategies to limit levels of glutamate by regulating glutamate metabolism may be new therapeutic targets in neurodegeneration.

3. Supplementation of nutrients and vitamins

Elevated plasma homocysteine (Hcys) has been associated with ocular complications including DR [35]. Homocysteine is a by-product of transmethylation reactions and is detoxified by methionine synthetase, which depends on vitamin B12 and folate as coenzymes for its proper function [36]. Low concentrations of folate and B-vitamin are also associated with an increased risk of diabetic complications [37]. Previously, we demonstrated that hyperglycemic and diabetic conditions reduce the expression and activity of the folate transporter and decrease the folate level in the diabetic retina [38]. Folic acid and vitamin B12 supplementation are known to reduce homocysteine levels [39]. Elevated homocysteine levels in both in vitro and in vivo models have shown to induce apoptosis in RGCs [40,41]. In vitro studies of RGC cells and in vivo studies in the brain suggest that homocysteine acts as an agonist at the glutamate site of NMDA receptors [42,43]. Other studies have also demonstrated that the neurotoxic effects of homocysteine are also associated with an activation of type II metabotropic glutamate receptors [44,45]. Therefore, strategies to control the level of homocysteine by supplementation with folic acid or vitamin-B12 may be potential treatment strategies to ameliorate neurodegeneration.

4. Metabolites of tryptophan pathway

Important metabolites in the kynurenine pathway generated by tryptophan degradation are thought to play an important role in neurodegenerative disorders. Among the important metabolites of the kynurenine pathway, 3-hydroxykynurenine and quinolinic acid are reported to have neurotoxic effects; however, kynurenic acid is a neuroprotectant [48]. Glutamate receptor-mediated excitotoxicity and free radical formation have been shown to correlate with decreased levels of the neuroprotective metabolite, kynurenic acid. It has been reported that homocysteine can alter the synthesis of kynurenic acid. It has been suggested that hyperglycemia
further increases the harmful effects of elevated homocysteine levels on the availability of kynurenic acid [46]. A recent study reported elevated levels of kynurenine, kynurenic acid and 3-hydroxykynurenine in the serum of DR patients [47]. Thus strategies to increase the level of kynurenic acid may protect neurodegeneration in diabetic retina.

5. Neurotrophic support

Retina is a neuronal tissue, it produces a substantial amount of neurotrophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4. Dysregulation of neurotrophic factors has been found to cause neurodegeneration and pathologic angiogenesis in DR [17, 50-52]. Diabetes progressively alters the level of trophic factors/signaling pathways in the retina, reducing the strength of survival signals and increasing apoptosis. Among neurotrophins, BDNF is produced by neurons and glial cells, which are known to maintain neuronal cells. The biological function of BDNF is mediated through high-affinity tyrosine kinase receptors [53]. Furthermore, BDNF has been shown to reduce damage to retinal ganglion cells after optic nerve lesions and to protect neurons in rodents under oxidative stress conditions [54,55]. In addition, BDNF also protects the retina from ischemic injury, promotes the survival of interneurons and plays an important role in the synaptic connections of a large number of neurons [56–58]. More recently, Min and coworkers suggested that BDNF provides a neuroprotective effect by increasing glutamate uptake and the upregulation of glutamine synthetase in Müller cells under hypoxic conditions [59]. In the animal model of diabetes, few studies showed that reduced levels of BDNF in the diabetic retina may damage neurons, [60,61]. Thus, regulating the level of BDNF in the diabetic retina may be a promising therapeutic target to protect neurons.

Several reports have documented increases in NGF levels in serum from diabetic neuropathy and retinopathy patients [49]. The increases in NGF levels positively correlated with the DR stage and other diabetes mellitus parameters. Several studies have indicated nerve growth factor (NGF) as a contributing factor to neurogenic inflammation and its association with hypoxia [62].

Glial derived neurotrophic factor (GDNF) is a member of the transforming growth factor-β (TGF-β)-related neurotrophic factor family. GDNF promotes photoreceptor survival during retinal degeneration that is mediated by the interaction of neurotrophic factors via receptors in Müller glial cells. Endogenous ciliary neurotrophic factor (CNTF) is upregulated in
response to retinal injury and may play an important role in neurodegeneration. Treatment with CNTF in combination with BDNF has been shown to rescue photoreceptors in retinal explants, thus conveying its neuroprotective effects [63].

Another important neurotrophic factor is PEDF, which offers neuroprotective properties [64]. PEDF protects neurons from glutamate mediated excitotoxicity. The level of PEDF is decreased in DR. Therefore, enhancing the expression of PEDF can be a therapeutic target to protect neurons.

In addition to the widely known role of VEGF in retinal vascularization, it has also been reported that VEGF is a potential neurotrophic factor. Endogenous VEGF has been found to be involved in the maintenance and function of retinal neurons. A study by Li group has shown that treatment with VEGF protects retinal ganglion cells in various models of neurotoxicity [65]. Therefore, it is important to consider neuroprotection, especially in the case of proliferative DR treatment using anti-VEGF to regress neovascularization.

Insulin has prosurvival actions in the retina and is considered as an important neurotrophic factor for retinal cells. In insulin-deficient diabetic rats, retinal death increased within a few weeks; and an increase in neuronal apoptosis has been observed in diabetic human eyes [2,66]. In the streptozotocin-induced diabetic rat model, it has been demonstrated that diabetes progressively impairs the constitutive retinal insulin receptor signaling pathway through Akt kinase and suggests that loss of this survival pathway may contribute to the initial stages of DR [67]. Gardner and coworkers have shown that insulin rescues retinal neurons from cell death in the diabetic rat retina; and intraocular injection of insulin restores insulin receptor activity [2,67]. Thus, longterm local delivery of insulin in the retina is needed to protect against neurodegeneration in DR [68].

6. Conclusions

A large body of evidences suggests that neurodegeneration is an early event in the diabetic retina. Increasing interest has been shown in protecting retinal neurons, especially retinal ganglion cells, which are vulnerable to be damaged by diabetes. Neurodegeneration in diabetic retina may implicate later in vascular damage. Numerous studies investigated mechanisms of neurodegeneration with the aim of uncovering a promising target for a successful neuroprotection. Diabetes-induced dysregulated levels of excitotoxic metabolites, altered neurotrophic support/signaling and oxidative stress are among the potential factors of neurodegeneration. Therapeutic
strategies to decrease the level of neurotoxic metabolites, increase the antioxidant status and to provide neurotrophic support may protect neurons in order to ameliorate the progression of DR, a leading cause of blindness worldwide.

Acknowledgement

The authors acknowledge support from Department of Biochemistry, College of Science at King Saud University.

References