The Pathogenesis of Diabetes and Its Negative Impact on the Retina: Diabetic Retinopathy

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Abstract

Diabetic retinopathy causes the plurality of blindness among the working class of America. This review focuses on the biochemistry and pathophysiology of the pancreatic cells during the development of diabetes as well as the effects that diabetes has on the Müller cells and pericytes of the retina at the cellular and molecular levels. In addition, the current research on some of the treatment options for diabetic retinopathy will be presented. This review is valuable to the scientific community because it presents a side-by-side summary of the pathogenesis of diabetes and diabetic retinopathy based on the current body of research.

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Introduction

Diabetes is on the rise in the United States, and the current trends suggest it is likely to continue rising in the coming years. The prevalence of diabetes in the United States increased from 9.5% to 12.0% between 2002 and 2016 (Center for Disease Control and Prevention, 2020). Furthermore, in 2018, an estimated 13.0% of American adults had diabetes, whether diagnosed or undiagnosed. Among other symptoms, diabetes can result in increased thirst, fatigue, muscle breakdown, and obesity. Because diabetes affects such a broad range of people in a variety of ways, researchers are constantly seeking to better understand the disorder and come up with new and more effective treatments. To create effective treatments, researchers must understand the pathogenic mechanisms of the two types of diabetes.

Diabetes results from damage to the cells of the pancreas and is divided into two main categories: type I diabetes and type II diabetes. In brief, type II diabetes is commonly associated with lifestyle choices such as a high-glucose diet which allows for consistently high concentrations of glucose in the blood. Type I diabetes, on the other hand, is the result of an autoimmune disorder in which the cells of the immune system attack and kill the cells of the pancreas. As will be described later, prolonged hyperglycemia causes damage and death to the cells of the pancreas. Without the proper functioning of the pancreatic cells, the body experiences an inability to produce the hormones necessary to regulate blood glucose levels. One of the most common symptoms of diabetes is diabetic retinopathy. Not only is diabetic retinopathy the most common complication of diabetes, but it is also the leading cause of blindness among working-age Americans (Lee et al., 2015; Wang & Lo, 2018). Similar to its

effects on the cells of the pancreas, hyperglycemia damages and kills the vascular and neural cells of the retina. This results in retinal hypoxia, ocular malnutrition, and further destruction of the retina. This review focuses on the causes and development of diabetes and pathology of diabetic retinopathy. In addition, treatment options and current research surrounding the diseased state will be discussed.

The Normal Functioning of the Pancreas and Blood Glucose Regulation

Blood glucose levels are tightly regulated to maintain homeostasis in the organism. Low levels of glucose in the blood, hypoglycemia, decreases the body's readily available nutrients and can result in shakiness, nausea, and decreased cognitive ability and awareness. Conversely, high levels of glucose in the blood, hyperglycemia, cause an increase in the body's metabolism which results in oxidative stress and subsequent damage to various cell types. The pancreas is the major regulatory organ of blood glucose levels. The endocrinal structures of the pancreas are referred to as the Islets of Langerhans and includes various cell types which play a role in maintaining blood glucose homeostasis.

The Cells of the Pancreas

The Islets of Langerhans are important endocrine structures involved in regulating various metabolic functions (Xavier, 2018). While the islets are composed of other cells such as pancreatic-polypeptide-hormone-releasing γ-cells, ghrelin-releasing ε-cells, and somatostatin-releasing δ-cells, the two major types of islet cells focused on in this review are the α-cells and β-cells. α-cells release glucagon to promote glycogen breakdown into glucose and increase blood glucose levels, while β-cells release insulin to promote glucose storage into glycogen and decrease blood glucose levels. By volume, α-cells and β-cells make up the majority of the

pancreatic islets. While γ-cells, ε-cells, and δ-cells combined account for roughly 10% of the Islets of Langerhans, the α-cells and β-cells represent 30% and 60% of the islets, respectively (Xavier, 2018). In addition to secreting hormones to adjust blood glucose levels, α-cells and β-cells are able to sense the concentration of glucose in the blood. The pancreas is highly vascularized so it can sense and respond to blood glucose levels in an accurate and timely fashion (Quesada et al., 2008). Blood passes through the β-cells which are closest to vascularization first, followed by the α-cells, and finally to the rest of the cells in the Islets of Langerhans. In order to accurately sense glucose concentrations in the blood, α-cells and β-cells must have a reliable means of transporting glucose from the blood into the cell.

Via facilitated diffusion, glucose transporters (GLUTs) within the plasma membranes of both α-cells and β-cells provide the ability to monitor the glucose concentration of the blood as it passes through the pancreas (Berger & Zdzieblo, 2020). In the β-cells, GLUT2 is the transporter primarily responsible for moving glucose across the plasma membrane. In contrast, GLUT1 appears to be the primary transporter of glucose across the membranes of α-cells. Facilitated diffusion of glucose across the membranes of α-cells and β-cells allows for an accurate measurement of blood glucose levels because facilitated diffusion relies solely on the concentration gradient. Therefore, the higher the concentration of glucose in the blood, the more glucose is transported through GLUT2 and GLUT1, and the higher the glucose concentration is inside the α-cells and β-cells, respectively. Although the initial means of glucose transport is very similar for both the α-cells and β-cells, each responds to glucose in a very different way.

Normal Functioning of Pancreatic β-Cells

In β-cells, the mechanism by which glucose signals for the release of insulin is dependent on fluctuations in the voltage potential across the membrane. In low blood glucose conditions (< 3 mM), antiport ion exchange pumps drive calcium (Ca^{2+}) ions out of the cell by using the concentration gradient of sodium (Na^{+}) ions (Gylfe, 2016; Klec et al., 2019). These Na^{+}-Ca^{2+} ion exchange pumps bring Na^{+} ions into the cell passively because the extracellular concentration of Na^{+} ions is much greater than the intracellular concentration. However, in order for Na^{+} ions to be transported via this exchange pump, the pump must bind Ca^{2+} ions from the inside of the cell and transport it outwardly. Thus, Ca^{2+} ions are driven out of the cell against their concentration gradient. At the same time, ATP-dependent potassium (K^{+}) ion channels are open and allow for facilitated diffusion of K^{+} ions from inside the cell to the outside of the cell along their concentration gradient. This outward flow of positively charged K^{+} ions allows the β-cells to reach and maintain a hyperpolarized resting membrane potential of -70 mV while the blood has relatively low levels of glucose (Rorsman & Ashcroft, 2018).

In hyperglycemic conditions (>5-7 mM), the process by which β-cells respond by secreting insulin starts with an increased transportation of glucose across the plasma membrane via GLUT2 transporters (Gylfe, 2016). The glucose is transported into the β-cells by facilitated diffusion along its concentration gradient. The higher the concentration of glucose in the bloodstream, the greater the concentration gradient and the more glucose is transported into the cell. Once the glucose has crossed the plasma membrane, it begins the process of glycolysis. According to Le Chatelier's principle, a chemical reaction at equilibrium will shift forwards or backwards in response to an excess of reactants or products, respectively (Rietman et al., 2013).

In the case of a sudden increase in intracellular glucose, glycolysis is no longer at equilibrium. Since glucose is a reactant in this reaction, the influx of glucose causes the reaction to shift strongly towards the product: ATP.

An increased ATP/ADP ratio affects ion channels embedded in the plasma membrane and ultimately releases insulin into the bloodstream. First, the greater ATP/ADP ratio inside the β-cells results in the closure of ATP-dependent K^+ ion channels (Jacobson et al., 2009). Without the constant flow of K^+ ions leaving the cell, the membrane potential becomes less negative because positively charged K^+ ions stay in the cell. Depolarization of the cell due to ATP-dependent K^+ ion channel closure causes L-type voltage-gated Ca^{2+} ion channels to open resulting in a sudden influx of Ca^{2+} ions along their concentration gradient (Klec et al., 2019). Ca^{2+} ions act as intracellular signals to initiate the transport of insulin-containing vesicles towards the plasma membrane. Research has demonstrated that a limited number of soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins (SNAREs) are responsible for the fusion of these vesicles with the plasma membrane. Namely, synaptobrevin-2, syntaxin-1, and SNAP-25 facilitate the exocytosis of insulin from β-cells (Röder et al., 2016). Synaptobrevin-2, lodged in the plasma membrane of the vesicle, binds to syntaxin-1 and SNAP-25 on the inner-surface of the cell's plasma membrane. These SNARE proteins remove the cytoplasm between the vesicle and the cell wall causing the fusion of phospholipid bilayers and the secretion of insulin into the bloodstream (Fasshauer et al. 1997).

In contrast, at a low concentration of glucose in the blood, β-cells have minimal amounts of glucose transported across the GLUT2 due to a smaller concentration gradient. Thus, glycolysis proceeds at a slower rate and the intracellular ATP/ADP ratio is relatively small. A

small ATP/ADP ratio allowed ATP-dependent K^+ ion channels to remain open and β-cells to remain polarized at a membrane potential of -70 mV (Röder et al., 2016; Rorsman & Ashcroft, 2018). At this membrane potential, there are no action potentials generated, no influx of Ca^{2+} ions, and therefore, no secretion of insulin.

Normal Functioning of Pancreatic α-Cells

α-cells, just as β-cells, respond to glucose via the opening and closing of ion channels and the rise and fall of action potentials. However, α-cells respond to glucose concentrations in a very different way compared to β-cells. In hypoglycemic conditions, GLUT1 transports less glucose into α-cells because there is a relatively low concentration gradient (Berger & Zdzieblo, 2020). Again, according to Le Chatelier's principle, low levels of glucose cause the glycolysis equilibrium to favor gluconeogenesis (Rietman et al., 2013). Therefore, less ATP is made and the ATP/ADP ratio is rather low. In such conditions, ATP-dependent K^+ ion channels are allowed to function with moderate activity facilitating the exit of K^+ ions along their concentration gradient (Quesada et al., 2008). The consistent flow of positively charged K^+ ions to the outside of the cell brings α-cells to an ideal membrane potential of about -60 mV. At this potential, T-type voltage-gated Ca^{2+} ion channels open and cause an influx of Ca^{2+} ions along their concentration gradient. This influx begins the depolarization of α-cells and initiates an action potential.

Once the Ca^{2+} ion influx from the T-type voltage-gated Ca^{2+} ion channels begins depolarization, voltage-gated Na^+ ion channels open and cause an influx of Na^+ ions to further depolarize the cell until it reaches a membrane potential between -40 and -30 mV (Quesada et al., 2008). At this membrane potential, voltage-gated L-type Ca^{2+} ion channels and voltage-gated N-type Ca^{2+} ion channels open. The L-type Ca^{2+} ion channels are the primary conduit of Ca^{2+}

ions and are therefore largely responsible for completing the action potential. The N-type Ca^{2+} ion channels on the other hand are needed to transport the Ca^{2+} ions that trigger the release of glucagon. Although the mechanism by which only the Ca^{2+} ions from N-type Ca^{2+} ion channels trigger release of glucagon is not fully understood, research demonstrates that only the N-type Ca^{2+} ion channels are involved in triggering exocytosis of glucagon (Gromada et al., 2004).

When the L-type Ca^{2+} ion channels have completed α-cell depolarization, voltage-gated A-type K^+ ion channels open and allow for the efflux of K^+ ions from the cell once again (Quesada et al., 2008). Since intracellular K^+ ion concentrations are relatively high, the K^+ ions rush outwards along their concentration gradient. This rapid efflux of positively charged K^+ ions repolarizes the cell until, with the help of open ATP-dependent K^+ ion channels, it once again reaches a membrane potential of -60 mV. At this stage, the T-type Ca^{2+} ion channels are poised to reopen and begin a new action potential. Thus, at low levels of glucose, action potentials are generated repeatedly to allow for the consistent release of glucagon until blood glucose concentration increase.

When blood glucose concentrations are elevated, GLUT1 transports greater amounts of glucose into the cell because of the increased concentration gradient. Glucose enters the process of glycolysis to generate more ATP and subsequently increase the ATP/ADP ratio. Greater amounts of ATP in α-cells result in closure of ATP-dependent K^+ ion channels so the cells are depolarized. Without the moderate activity of ATP-dependent K^+ ion channels allowing for the efflux of K^+ ions, the α-cells settle at a resting membrane potential which is less negative than -60 mV (Quesada et al., 2008). At this membrane potential, T-type Ca^{2+} ion channels are rendered inactive and the cell is unable to produce an action potential. Without the opening of T-

type Ca^{2+} ion channels, α-cells are not depolarized, N-type Ca^{2+} ion channels remain closed, and glucagon exocytosis is not initiated. Therefore, high blood glucose concentrations inhibit the release of glucagon from α-cells.

The Pathogenesis of the Diabetic Pancreas

Pathogenesis in Type II Diabetes

Under certain circumstances, the glucose dependent regulation of blood glucose levels is disrupted and homeostasis is disturbed. Diabetes is characterized by hyperglycemia; however, not only does diabetes cause hyperglycemia, but in the case of type II diabetes, hyperglycemia can cause diabetes (Cerf, 2013). As described above, hyperglycemia causes an increased intracellular concentration of glucose in α-cells and β-cells. In controlled amounts, this is a good and normal process. However, chronic hyperglycemia causes overstimulation of mitochondria, dysfunction of insulin and glucagon secretion, and cellular death.

Increased intracellular glucose concentrations stimulate an increased rate of glycolysis which reduces intracellular glucose concentration while raising levels of pyruvate. Pyruvate is transported to the mitochondria and converted to Acetyl-CoA to be used in the Kreb's cycle. The Kreb's cycle uses Acetyl-CoA to generate high energy electrons in the form of NADH and FADH. These high energy electrons are used in the electron transport chain to generate the major source of cellular energy, ATP. Naturally, the electron transport chain generates reactive oxygen species (ROS). In a state of chronic hyperglycemia, however, overstimulation of the electron transport chain creates elevated levels of ROS which damage and kill α-cells and β-cells. Furthermore, many researchers conclude the islets of the pancreas are especially susceptible to oxidative stress because they produce less antioxidants compared to other organs (Cerf, 2013;

Kim et al., 2017). To counter the dangerous effects of oxidative stress, α-cells and β-cells respond in different ways.

ROS are multipurpose intracellular signals. ROS can signal for cell proliferation as well as cellular death (Kim et al., 2017). These opposite effects to the same signal depend largely on the cell's ability to upregulate phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt). If the cell is able to upregulate PI3K and Akt, such as in α-cells, then increased cell proliferation is signaled. In contrast, β-cells are unable to activate PI3K and Akt signaling. Instead, β-cells activate Forkhead box gene, group O (FoxO) in an attempt to resist oxidative stress. FoxO is a transcription factor that causes increased expression of antioxidant genes, especially manganese superoxide dismutase (MnSOD), to counter the effects of oxidative stress. To summarize, α-cells respond to ROS by secreting proliferation factors and replacing the cells induced to apoptosis by ROS. β-cells, on the other hand, increase transcription of antioxidants in an effort to counter the oxidative stress imposed by ROS.

In prolonged hyperglycemic states, the α-cells' method of survival outcompetes that of the β-cells. Although β-cells produce more antioxidant, they are unable to produce enough to counter rapid production of ROS via the electron transport chain (Kim et al., 2017). Furthermore, research demonstrates that hyperglycemia inhibits β-cell proliferation, so they are unable to proliferate at a rate fast enough to counter the increased rate of apoptosis due to ROS damage. Thus, β-cells die at a much faster rate than do α-cells, and consequently, α-cells make up a greater percentage of the pancreatic islets. β-cell death is responsible for many of the downstream implications involved with diabetes. The pathogenesis of type I diabetes involves

the death of β-cells, too. However, the process by which β-cells die in type I diabetes is much different than in type II diabetes.

Pathogenesis in Type I Diabetes

Clearly, hyperglycemia has detrimental effects on the pancreas. Hyperglycemia is a major concern for people with type I diabetes as well as in individuals with type II diabetes; however, the pathogenesis of type I diabetes is quite different from that of type II diabetes. While in type II diabetes, β-cell death is caused by oxidative stress in response to hyperglycemia, type I diabetes is an autoimmune disorder in which β-cells are killed by the cells of the immune system. Any time the pathogenesis of an autoimmune disease is considered, it is necessary to think about the topic of immune tolerance. Tolerance, the ability of the immune system to eliminate or suppress auto-reactive immune cells, can be broken down into two main categories: central tolerance and peripheral tolerance. Defects in either of these systems can lead to the development of an autoimmune disease.

In some cases, β-cell autoreactivity is the result of T cells evading central tolerance. For some patients with type 1 diabetes, the *Autoimmune Regulator* (*AIRE*) gene is not present or is not functioning at normal levels due to genetic inheritance (Cheng & Anderson, 2013). Many pancreatic autoantigens such as proinsulin are dependent on *AIRE* for their expression by medullary thymic epithelial cells (mTECs) in the thymus. Therefore, in people with less than adequate or with no expression of the Aire protein, mTECs are unable to negatively select autoreactive T cells. These autoreactive T cells are allowed to leave the thymus and enter circulation where they make their way to susceptible β-cells and kill them. In addition to central tolerance, peripheral tolerance is typically impaired in individuals who develop type I diabetes

(Kwong et al., 2021). Multiple studies have demonstrated that patients with type I diabetes exhibit decreased levels of regulatory T (Treg) cells which inhibit their ability to suppress the immune response against autoantigens (Atkinson et al., 2014; Graham et al, 2012). Without the proper response of Treg cells to suppress immune function, autoreactive T cells are allowed to kill β-cells without regulation. This also helps to explain why β-cell death occurs much more rapidly in type I diabetes compared to type II diabetes (Sarikonda et al., 2014). Whether by failure of central tolerance or peripheral tolerance, the immune cells of patients with type I diabetes attack and kill β-cells.

In some instances, type I diabetes is brought on by environmental factors instead of genetic factors. One common theory is that environmental factors could cause initiation of mild, controlled inflammation which could progress to uncontrolled, chronic inflammation and autoimmunity (Bettini & Vignali, 2011). For example, viral infection with a pathogen that affects the pancreas could cause T cells to rightfully attack β-cells in order to stop the infection. As the β-cells die, the result is mild, controlled inflammation of the pancreas. However, in some cases, allowing T cells to attack the β-cells of the pancreas could create memory T cells that recognize components of β-cells. After the initial infection, one of these memory cells may recognize and attack the β-cells which would create a stronger immune response than before. Thus, pathogen infection can lead to type I diabetes because of normal immune function.

The Consequences of β-cell Death

The death of β-cells and concurrent survival of α-cells creates problems for blood glucose regulation. First, with decreases in the number of β-cells, insulin secretion is impaired and glucose uptake from the blood is decreased (Cerf, 2013). This leaves more glucose in the blood

and contributes to the hyperglycemic state. In addition, many researchers call diabetes a "bi-hormonal" disease, because abnormal levels of both insulin and glucagon secretions contribute to hyperglycemia (Kim et al., 2017). In fact, some research demonstrates that impaired insulin secretion may cause dysfunction of glucagon-secreting α-cells. The secretion of glucagon by α-cells is dependent not only on blood glucose levels, but also on insulin secretion by β-cells (Cryer, 2012). In the properly functioning pancreas, the rise and fall of insulin secretion is inversely correlated to the secretion of glucagon. Thus, elevation in insulin secretion inhibits glucagon secretion, and cessation of insulin secretion stimulates glucagon secretion. In diabetes, it is observed that the decreased insulin secretion due to apoptosis of β-cells stimulates an increase in glucagon secretion from α-cells despite high levels of glucose in the blood. In turn, glucagon release stimulates the liver to break down glycogen into glucose to be released into the bloodstream furthering the problem of hyperglycemia. At this point in the progression of both type I and type II diabetes, the pancreas loses regulatory ability over blood glucose concentrations and the body experiences a chronic state of hyperglycemia.

The Normal Functioning of Retinal Cells

As briefly described above, diabetes has a wide range of negative implications for the body. Of these negative implications, diabetic retinopathy is the most common microvascular complication in patients with diabetes (Wang & Lo, 2018). Chronic hyperglycemia has detrimental effects on the cells of the retina. Specifically, diabetes causes dysfunction and death of retinal Müller cells and pericytes.

Müller Cell Function

Müller cells perform various functions in the retina including regulating immune responses, regulating inflammation, structurally stabilizing the retina, and promoting the survival of photoreceptors and neurons by secreting trophic substances and removing metabolic waste (Bringmann & Wiedemann, 2012). In addition to these important functions, Müller cells are responsible for uptake of glutamate and production of glutathione (Bringmann & Wiedemann, 2012; Li & Puro, 2002; Muller et al., 1998). Glutamate is the major excitatory neurotransmitter of the retina and can excite more than 90% of the synapses in the retina (Li & Puro, 2002; Muller et al., 1998). Not only are Müller cells needed for the proper function of synapse excitation, but retinal neurons are also dependent on Müller cells for their survival. Without the function of Müller cells to remove glutamate from the retina, prolonged excitation of glutamate receptors kills retinal neurons. In fact, glutamate toxicity is one of the primary mechanisms by which neuronal cells die in many retinal disorders (Bringmann & Wiedemann, 2012).

Müller cells are responsible for the uptake of glutamate to ensure proper neuronal signaling and prevent cell death from overexcitation. In addition, Müller cells use glutamate to produce glutathione which is a vital antioxidant. The process of synthesizing glutathione requires Müller cells to transport glutamate, cysteine, and glycine intracellularly from their surroundings (Bringmann & Wiedemann, 2012). Glutathione is a critical mediator in the hostile environment of the retina because the retina produces more oxidative stress than any other place in the body (Kowluru & Chan, 2007). One potential reason why the retina is more susceptible to oxidative stress is that it uses a large amount of energy and therefore has a higher oxygen uptake and

glucose oxidation than any other tissue. Glutathione is the retina's main defense against these extreme amounts of oxidative stress and Müller cells are primarily responsible for its production.

Pericyte Function

The primary responsibility of pericytes is to surround and stabilize the microvasculature of the retina. Pericytes have finger-like extensions that intertwine with each other to form a stable network of cells surrounding the blood vessels. In the development of the retina, pericytes perform important functions such as regulation of endothelial cell growth (Ejaz et al., 2007). When the eye is developing, pericytes release pro-angiogenic cytokines which signal endothelial cells to proliferate so the retina is properly vascularized to receive the nutrients necessary for the high-energy functions it must perform. Once pericytes have matured, they become more angiostatic in nature to prevent further proliferation of endothelial cells and prevent the development of new blood vessels (Kremer et al., 2020). Clearly, pericyte function is vital to a healthy retina.

The Pathogenesis of the Diabetic Retina

Diabetic retinopathy is a disorder of the retina that primarily affects the microvasculature (Wang & Lo, 2018). However, if left unchecked, the problems involved in a diabetic retina can lead to neuronal cell death and eventually blindness. Diabetic retinopathy can be classified into two categories. Non-proliferative diabetic retinopathy is the early stage of the disease and is commonly characterized by increases in vascular permeability and capillary occlusions. The latter of the two can lead to microaneurysms and hemorrhages. Despite the extensive turmoil of the inner eye, patients with non-proliferative diabetic retinopathy are typically asymptomatic at this point in the disease. Upon progression of the diseased state, the patient enters the second

stage of diabetic retinopathy which is called proliferative diabetic retinopathy. This stage of the disease is more advanced and is commonly characterized by angiogenesis. Angiogenesis is the formation of new blood vessels and is common in the pathology of diabetic retinopathy as well as other diseases such as cancer and psoriasis (Paulas & Sodhi, 2017). In proliferative diabetic retinopathy, angiogenesis is dangerous for multiple reasons mainly pertaining to the structural integrity of the new blood vessels as well as their lack of directional growth. Blindness is the major concern and ultimate result of damage to the retina; therefore, it is important to understand how diabetic retinopathy damages the specific cells of the eye.

Hyperglycemia and Müller Cells

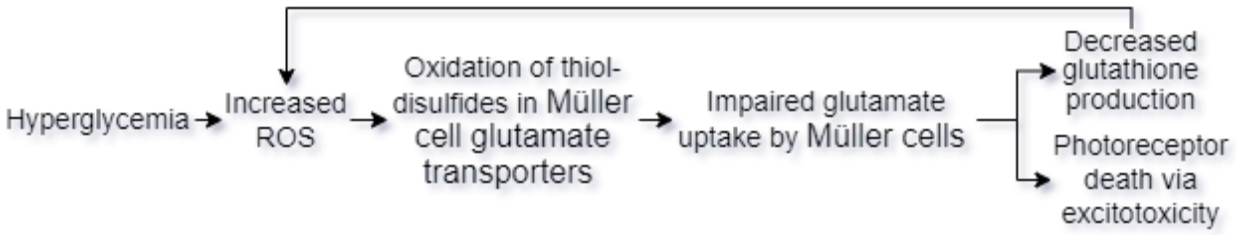
As is the threat to most cells in hyperglycemic conditions, the cells of the retina are endangered by the increased presence of ROS. In a similar way to α-cells and β-cells, the increased production of ROS in the retina is because of overstimulation of the mitochondria. Therefore, glucose is metabolized at a greater rate due to the excessive amounts of intracellular glucose. The primary contributor to ROS in the retina is the photoreceptor cells which have more mitochondria compared to any other cell type in the retina (Kang & Yang, 2020). One of the main cell types affected by increased ROS are Müller cells.

Hyperglycemia can cause Müller cell dysfunction by impairing transporter function (see Figure 1). As described above, Müller cells are responsible for the uptake of glutamate and the production of the major antioxidant glutathione. An aspect of Müller cells that makes them well suited for this function is that they have redox-sensing glutamate transporters (Li & Puro, 2002). Under normal conditions, this transporter regulates the amount of glutamate uptake via thiol-disulfide redox interconversions. In hyperglycemic conditions, however, glutamate transport is

disrupted which has major implications for the retina and specifically the Müller cells. When studying the glutamate transporter of Müller cells, the research team of Muller et al. (1998) observed a dose-dependent decrease in glutamate uptake by Müller cell transporters in response to hydrogen peroxide. In addition, research done by Li and Puro (2002) demonstrated a 67% decrease in transporter activity in the Müller cells of mice thirteen weeks after the development of diabetes. Furthermore, in this same study, Li and Puro found that treating the Müller cells with disulfide-reducing agents fully and rapidly restored the glutamate transporter activity.

Figure 1

Hyperglycemia Causes Müller Cell Dysfunction



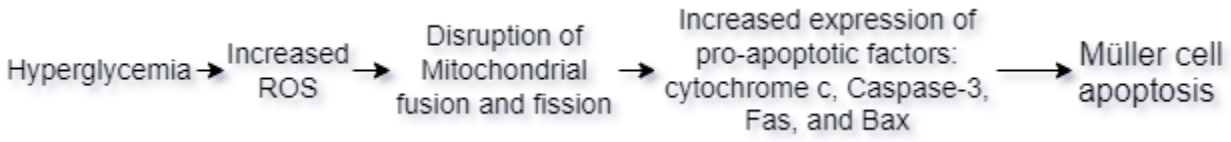
The summation of these studies indicates two things. First, efficiency of glutamate transport in Müller cells is directly affected by the oxidative conditions of the retina. Second, excessive amounts of oxidative stress lead to transporter dysfunction via oxidation of thiol-disulfides. Consequently, transporter dysfunction further damages the retina by decreasing glutamate uptake. The increased concentration of glutamate in the retinal environment causes neural cell apoptosis via excitotoxicity (Bringmann & Wiedemann, 2012). As mentioned before, glutamate is the major excitatory neurotransmitter in the retina and excessive stimulation due to a lack of glutamate uptake is one of the most common causes of neuronal cell death in retinal diseases. Glutamate transporter dysfunction not only causes damage to the retina due to a decrease in glutamate uptake by the Müller cells, but also due to a decrease in glutathione

production. Since glutathione is the major antioxidant of the retina, a decrease in its production only exaggerates the problem of oxidative stress. This puts the retina into a positive feedback system in which elevated ROS decrease glutathione production causing further elevation of ROS.

Oxidative stress affects Müller cells by shutting down their glutamate transporters, but it also induces Müller cell apoptosis (see Figure 2). In addition to mitochondrial overstimulation, hyperglycemic conditions cause mitochondrial dysfunction (Tien et al., 2017). Inside the cell, mitochondria are constantly colliding and combining to form bigger mitochondria as well as splitting to form smaller mitochondria. This process is called fusion and fission, respectively. Some research suggests that high glucose levels disrupt mitochondrial fusion and fission. According to the research done by Tien et al. (2017), when exposed to high glucose levels, Müller cells' mitochondria demonstrated increased mitochondrial fragmentation as well as decreased oxygen consumption.

Figure 2

Hyperglycemia causes Müller cell apoptosis



Upon dysfunction of the mitochondria, cells are programmed to induce apoptosis. This is done by the release of pro-apoptotic markers. In the same research done by Tien et al. (2017), mitochondrial dysfunction was observed concurrently with a significant increase in cytochrome c release. Cytochrome c is released from the mitochondria to signal the cell to induce apoptosis. Moreover, cytochrome c is not the only pro-apoptotic marker observed in Müller cells when

exposed to high glucose concentrations. Caspase-3, Fas, and Bax have all been found in elevated amounts in the diabetic retina (Abu El-Asrar et al., 2004). The combination of these apoptotic markers makes it clear that high glucose is inducing Müller cell death. Apoptosis of Müller cells, like glutamate transporter dysfunction, poses serious risk to the other cells of the retina. Due to Müller cell apoptosis, the retina has fewer cells able to remove glutamate, synthesize glutathione, release trophic factors, and remove metabolic waste. Importantly, with fewer Müller cells, there is less support for the pericytes surrounding the retinal microvasculature.

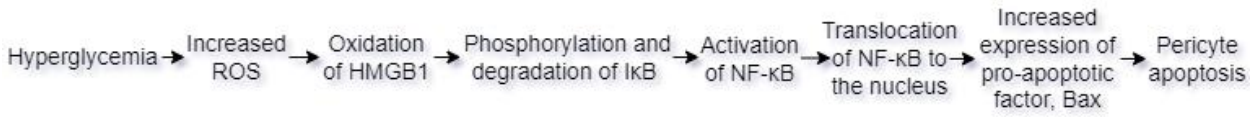
Hyperglycemia and Retinal Pericytes

Oxidative stress is the driving force of diabetic diseases. Oxidative stress causes apoptosis in α-cells, β-cells, and Müller cells, and as could be predicted, oxidative stress causes apoptosis in retinal pericytes (see Figure 3). One way that oxidative stress signals for apoptosis in pericytes is by causing the activation of nuclear factor κB (NF-κB) which is a transcription factor for important pro-apoptotic and pro-inflammatory genes (Romeo et al., 2002). The mechanism by which ROS lead to activation of NF-κB includes activity from high mobility group box 1 (HMGB1) (Nebbioso et al., 2020). HMGB1 is equipped with redox sensing ability via the presence of disulfide bridges and its function is dependent on the redox state of its immediate environment. As oxidative stress increases in response to hyperglycemia, HMGB1 becomes oxidized from its reduced form into the disulfide form. In its disulfide form, HMGB1 is able to activate NF-κB. When inactivated, NF-κB is bound by inhibitory κB proteins (IκB) (Romeo et al., 2002). In its disulfide state, HMGB1 is able to direct phosphorylation and subsequent degradation of IκB. Without this inhibitory molecule, NF-κB is free to translocate to

the nucleus of the cell where it increases expression of pro-apoptotic factors such as Bax and pro-inflammatory factors such as TNF-α (Suryavanshi & Kulkarni, 2017).

Figure 3

Hyperglycemia Causes Pericyte Apoptosis



Pericyte apoptosis is extremely dangerous to the diabetic retina. Most researchers agree that pericytes in the adult retina do not replicate, so there is only a finite number of pericytes in the eyes of each person (Romeo et al., 2002). Interestingly, oxidative stress in the retina does not have the same effect on vascular endothelial cells as it does pericytes. Although the pericytes surrounding and supporting them are subject to apoptosis, the number of endothelial cells remains constant. As an estimate, the ratio of pericytes to endothelial cells in the adult retina is 1:1 (Kowluru & Chan, 2007). In the diabetic retina, on the other hand, this ratio is reduced to 1:4 indicating a severe loss of pericytes with no significant change in the number of endothelial cells. However, without the support of pericytes, the microvasculature of the retina is subject to various kinds of damage.

Downstream Effects of Pericyte Apoptosis

Pericyte apoptosis leaves gaps in the support of blood vessels. In the sensitive microenvironment of the retina, these small gaps of support put stress on the endothelial cells and normal blood pressure can cause outpouching of the vessels (Wang & Lo, 2018). Outpouching of the blood vessels commonly leads to blood vessel rupture and consequent retinal bleeding and lipid deposits. Furthermore, in addition to activating Bax, NF-κB causes the release

of TNF-α from pericytes (Romeo et al., 2002). As a cytokine, TNF-α can change endothelial cell phenotype so they exhibit pro-inflammatory and pro-coagulant properties. These properties may lead to capillary occlusions and further rupturing of the blood vessels. Excessive bleeding in the retina causes neuronal cell death by glutamate excitotoxicity (Li & Puro, 2002). Typically, the microenvironment of the retina has a relatively low concentration of glutamate because of the action of Müller cells as described above. Blood, on the other hand, has a high concentration of glutamate. When capillary occlusion or outpouching of the vessels leads to retinal bleeding, the influx of blood greatly increases the retinal concentration of glutamate which kills the neuronal cells of the retina by overexcitation.

Retinal blood vessel rupture causes damage to the eyes in other ways, too. For example, one common symptom of diabetic retinopathy is diabetic macular edema (Wang & Lo, 2018). Diabetic macular edema arises from breakdown of the blood retinal barrier and is the most common cause of vision loss in patients with diabetic retinopathy (Duh et al., 2017). Typically, loss of vision due to diabetic macular edema is the first noticeable symptom in patients with diabetic retinopathy. The macula is the central portion of the retina and is responsible for receiving light for perception of entities in sharp focus. When blood vessel permeability is increased or blood vessels rupture, it can cause a fluid accumulation underneath the macula disrupting vision by multiple mechanisms. First, fluid accumulation causes swelling of the macula which disrupts the normal path of light from the surface of the retina to the photoreceptors underneath (Daruich et al., 2018). Furthermore, the introduction of fluid to the macula changes the refractive index of retinal layers. In short, light travels at different speeds though different mediums. Fluid in the macula changes the speed by which light travels and

therefore alters its path to the photoreceptors. For these reasons, patients with diabetic macular edema often report experiencing metamorphopsia (Musat et al., 2015). This type of visual impairment manifests itself by distorting images so straight lines seem curved.

Beyond diabetic macular edema, damage to blood vessels yields a decreased transportation of nutrients such as oxygen. Less oxygen yields a further increase of ROS because there is no recipient for the high-energy electrons from the electron transport chain. Yet, the retina is affected by decreased oxygen transportation in more significant ways. At this stage in the disease, the hallmark of proliferative diabetic retinopathy becomes evident, which is angiogenesis (Al-Kharashi, 2018). In response to hypoxia and damage to the microvasculature, endothelial cells release vascular endothelial growth factor (VEGF) which signals for an increase in vascular permeability as well as endothelial cell proliferation. VEGF causes endothelial cells to first secrete matrix metalloproteases (MMPs) which degrade the surrounding basement membrane. New endothelial cells are then allowed to migrate away from their parent cells into the gaps where the basement membrane has been degraded. Using adhesive molecules such as integrins, these new endothelial cells begin proliferation in the extracellular matrix. It is this proliferation that results in the formation of a network of new blood vessels.

Although angiogenesis is beneficial in some cases, such as the development of the retina, angiogenesis in the adult diabetic is one of the main components of proliferative diabetic retinopathy. First, the new blood vessels do not fix the problem for which they were developed. New blood vessels are formed without a sense of direction. Therefore, most of them do not reach the malnourished areas of the retina that signaled for angiogenesis in the first place (Fadini et al., 2019). Moreover, the newly formed vessels are weaker than the parent vessels and more likely to

rupture causing further damage to the retina via glutamate excitotoxicity. Rupturing of these new vessels could potentially cause vision-decreasing hemorrhages in the vitreous humor of the eye as well. Excessive glutamate excitotoxicity and prolonged hypoxia can culminate in death of neuronal cells significant enough to cause blindness.

Furthermore, the new blood vessels occasionally invade the vitreous humor and cause tractional retinal detachments (Fadini et al., 2019). When the vitreous is disrupted by the growth of new blood vessels, it moves and pulls part of the retina with it causing severe damage to the retina and potentially blindness. Finally, in some cases, the new vasculature grows into the angle of the anterior chamber of the eye and obstructs the outflow of vitreous fluid. This causes an accumulation of fluid inside the eye leading to increased intraocular pressure. This pressure damages the optic nerve and progressively kills nerve cells from the outer portion of the retina inwardly. This specific type of damage to the optic nerve is called neovascular glaucoma and can also lead to blindness. Loss of retinal pericytes disrupts retinal homeostasis in a variety of ways.

Outlook for Diabetic Retinopathy

Diabetic retinopathy is a serious concern for an increasingly large population of Americans. For this reason, it is important to understand the best treatment options as well as the potential for better means of treatment in the future.

Current Treatment of Diabetic Retinopathy

When treating diabetic retinopathy, the first step is to target the source of the problem: hyperglycemia. Traditionally, blood glucose levels are lowered by drugs such as metformin, which acts as an insulin sensitizer, or by exogenous insulin administration, which is typically done later in the progression of diabetes. For patients ranging anywhere from pre-diabetic to

having elevated blood sugar for a decade or more, metformin is among the most commons drugs prescribed (Viollet et al., 2012). Metformin is valuable because of its ability to lower blood glucose levels without causing hypoglycemia. One of the main roles of metformin in the reduction of blood glucose levels is to act as an insulin sensitizer. By upregulating expression of the insulin receptor in the body's tissues, metformin causes increased insulin sensitivity and glucose uptake. Consequently, blood glucose concentrations are better regulated.

Further downstream in the progression of diabetes, exogenous insulin administration may be utilized to lower glucose concentrations in the blood; however, doctors typically do not start this type of treatment until ten to fifteen years after diagnosis with diabetes (Swinnen et al., 2009). The reason physicians and patients are wary to start exogenous insulin therapy is because it can cause hypoglycemia and weight gain. Furthermore, the administration involves painful injections that must be done on a regular basis. Although there are clear drawbacks to exogenous insulin therapy, studies have demonstrated great benefits from using insulin early in the treatment of diabetes. Early insulin therapy has beneficial effects on the β-cells of the pancreas (Weng et al., 2008). Studies suggest that early insulin administration improves β-cell function and has positive implications on patient remissions. Moreover, early insulin therapy benefits the retina. Usage of insulin within the first month of diagnosis with diabetes results in fewer apoptotic cells in the retina (Barber et al., 1998). Metformin and insulin are both beneficial therapies for treating the root of diabetic retinopathy, but other treatment options are used to directly ameliorate damage to the retina.

In treatment of diabetic retinopathy, by far the most widely used therapy is anti-VEGF therapy. The goal of anti-VEGF therapy is to stop angiogenesis and thus prevent further damage

to the retina through hemorrhages, retinal detachments, and neovascular glaucoma. Two of the most common anti-VEGF agents are ranibizumab and bevacizumab; both work in similar ways. Ranibizumab is a recombinant, humanized, monoclonal antibody fragment with a high affinity to VEGF (Gaudreault et al., 2005). Likewise, bevacizumab is a humanized immunoglobulin G antibody with a high affinity to VEGF (Krämer & Lipp, 2007). Both antibodies bind to VEGF in the circulation of retinal microvasculature so it cannot bind to its receptor to promote angiogenesis. The downside of using these anti-VEGF agents is that ranibizumab and bevacizumab have half-lives of approximately 3 days and 20 days, respectively (Gaudreault et al., 2005; Krämer & Lipp, 2007). Thus, the agents, which are administered by intravitreal injections, must be given weekly, biweekly, or triweekly.

The next most common treatment for the diabetic retina is laser photocoagulation. Photocoagulation techniques were one of the first treatments of diabetic retinopathy, and although this treatment option is used less frequently since the advent of anti-VEGF therapy, it is still used somewhat commonly as a viable means to treat the diabetic retina. Laser photocoagulation works by applying focused, high-energy light to specific regions of the retina to cauterize broken blood vessels or kill small regions of retinal cells (Everett & Paulas, 2021). Cauterizing broken blood vessels stops retinal bleeding. Killing retinal cells in areas of poor perfusion decreases the demand for oxygen and other nutrients in that portion of the retina thereby reducing oxidative stress and VEGF secretion. In many cases, the brain is able to ignore the gaps in photoreceptive information and still produce a reliable image. However, in some cases, laser photocoagulation has caused permanent damage to the retina resulting in mild central vision loss and reduced night vision (Wang & Lo, 2018). Treatments for diabetic retinopathy are

beneficial in many ways, but further research is needed to discover treatment options without serious drawbacks.

Potential Treatment Options for Diabetic Retinopathy

Because diabetic retinopathy is such a prevalent and prominent complication of diabetes, researchers are constantly searching for new treatment options. Among the most recent research, some of the promising potential treatments for diabetic retinopathy include inhibiting fatty acid synthase (FASN) to prevent angiogenesis and treatment with adipose-derived stromal cells (ASC) as a means of repairing the retinal microenvironment.

Research done by Bruning et al. (2018) sought to determine the effects of FASN inhibition on angiogenesis. An analysis of endothelial cells with a FASN knockout as well as after treatment with the FASN inhibitor orlistat demonstrated that decreased FASN activity affected endothelial cell proliferation and migration. This was done by the use of a scratch wound migration assay and a spheroid capillary sprouting assay. In the former, the research team reported minimal changes in migration due to FASN knockout or inhibition. In the latter, however, there was a significant decrease in the endothelial cell proliferation in response to FASN knockout and inhibition. From this data, the researchers concluded that FASN inhibition causes a decrease in angiogenesis by impairing endothelial cell proliferation. This research informs the scientific community of a potential use of FASN inhibition in the treatment of diabetic retinopathy.

Other researchers have explored the potential of using ASC as a means to cure the diabetic retina. Kremer et al. (2020) conducted research to compare ASC to retinal pericytes. Before this research was done, other studies in diabetic retinopathy models had demonstrated

that ASC therapy could potentially provide a cytoprotective and reparative environment for damaged endothelial cells and pericytes. ASC have anti-inflammatory and anti-apoptotic properties to help slow progression of diabetic retinopathy. In addition, ASC integrate themselves into the retina to replace detached pericytes and provide support for the microvasculature. Despite all these benefits, clinical trials have not been completely successful, so Kremer and his colleagues conducted research to further the scientific community's understanding of ASC.

To explore the potential for ASC therapy in diabetic retinopathy, the research team first analyzed the adhesion of both pericytes and ASC to endothelial cells in high-glucose conditions (Kremer et al., 2020). The result was that high-glucose impaired pericyte adhesion but had no effect on ASC adhesion. In addition, Kremer and his team set up an angiogenesis assay to compare angiogenic properties of ASC to pericytes. In contrast to pericytes which had angiostatic properties, ASC caused angiogenesis in endothelial cells similarly to the positive control, VEGF. Finally, Kremer and colleagues tested the effects of VEGF receptor 2 (VEGFR-2) inhibitor ZM 3238811. In the presence of ZM 3238811, ASC angiogenic activity was decreased indicating that the pro-angiogenic activity of ASC is mostly due to VEGF signaling. The results of this research suggest that clinical trials may not have been successful due to the angiogenic activity of ASC. Moreover, this study provides reason to pursue ASC therapy in combination with anti-VEGF therapy as means to treat diabetic retinopathy.

Conclusion

As diabetes is continuing to affect more people every year, the number of people who develop diabetic retinopathy will only increase in the foreseeable future. For this reason, the

importance of understanding this disease's pathogenesis as well as the potential for therapy cannot be stressed enough. Although diabetic retinopathy has serious implications for the eyes, researchers have found viable treatment options to ameliorate symptoms. Furthermore, current research seems to hold great potential for even better treatment options in the not-too-distant future. The pathogenesis of diabetic retinopathy progressing from the disruption and apoptosis of α-cells and β-cells in the pancreas leading to dysfunction and apoptosis of Müller cells and pericytes in the retina truly is an intricate and intriguing process.

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