World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v6.i2.312

World J Diabetes 2015 March 15; 6(2): 312-320
ISSN 1948-9358 (online)

MINIREVIEWS

Current knowledge on diabetic retinopathy from human donor tissues

Jessica H Eisma, Jennifer E Dulle, Patrice E Fort

Jessica H Eisma, Jennifer E Dulle, Patrice E Fort, Departments of Ophthalmology and Visual Sciences, Kellogg Eye Center, University of Michigan, Ann Arbor, MI 48105, United States

Author contributions: Eisma JH wrote the first draft of the paper; Dulle JE and Fort PE edited, corrected and formatted the manuscript.

Conflict-of-interest: Patrice E Fort has received research funding from the National Eye Institute (NIH-NEI); Patrice E Fort is an employee the University of Michigan; none of the authors have any conflict of interest.

Correspondence to: Patrice E Fort, PhD, Departments of Ophthalmology and Visual Sciences, Kellogg Eye Center, University of Michigan, 1000 Wall Street, Ann Arbor, MI 48105, United States. patricef@umich.edu
Telephone: +1-734-2328225
Fax: +1-734-2328030

Received: September 17, 2014
Peer-review started: September 20, 2014
First decision: December 17, 2014
Revised: December 23, 2014
Accepted: December 29, 2014
Article in press: December 31, 2014
Published online: March 15, 2015

Abstract

According to the American Diabetes Association, diabetes was the seventh leading cause of death, and diabetic retinopathy the leading cause of blindness in working age adults in the United States in 2010. Diabetes is characterized by hyperglycemia associated with either hypoinsulinemia or insulin resistance, and over time, this chronic metabolic condition may lead to various complications including kidney failure, heart attacks, and retinal degeneration. In order to better understand the molecular basis of this disease and its complications, animal models have been the primary approach used to investigate the effects of diabetes on various tissues or cell types of the body, including the retina. However, inherent to these animal models are critical limitations that make the insight gained from these models challenging to apply to the human pathology. These difficulties in translating the knowledge obtained from animal studies have led a growing number of research groups to explore the diabetes complications, especially diabetic retinopathy, on tissues from human donors. This review summarizes the data collected from diabetic patients at various stages of diabetic retinopathy and classifies the data based upon their relevance to the main aspects of diabetic retinopathy: retinal vasculature dysfunction, inflammation, and neurodegeneration. This review discusses the importance of those studies to discriminate and establish the relevance of the findings obtained from animal models but also the limitations of such approaches.

Key words: Retina; Diabetic retinopathy; Human donor; Physiopathology; Vascular disease; Inflammation; Neurodegeneration

Core tip: This review summarizes the current state of the knowledge on the physiopathology of diabetic retinopathy directly obtained from the analysis of tissues from human patients strongly complementing what has been gathered from animal models. The review discusses the vascular, inflammatory and neurodegenerative aspects of the disease, their inter-relation and the advantages and limits of such studies compared to the ones using animal models. Altogether, these analyses clearly demonstrate the complexity of the disease mechanisms but also the somewhat

still limited knowledge and the need for additional complementary studies.

Eisma JH, Dulle JE, Fort PE. Current knowledge on diabetic retinopathy from human donor tissues. *World J Diabetes* 2015; 6(2): 312-320 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i2/312.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i2.312

INTRODUCTION

Diabetic retinopathy (DR) is one of the most prevalent complications of diabetes, and while it is now well recognized that this disease involves perturbations of all the components of the retinal tissue, its diagnosis still mostly relies on the detection of damages associated with the vascular network. Based on those clinical features, diabetic retinopathy has been subdivided into two main stages: non-proliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR). In the primary stages of NPDR, microaneurysms, or the swelling of small blood vessels, are the first detectable clinical signs of DR, and are associated with hypoperfusion of discrete regions of the retina. While the clinical gradation is still made based on vascular pathology, recent studies have shown that ganglion cell sensitivity as well as dark adaptation is altered in patients with NPDR, thus confirming early alteration of neuronal function[1] and raising questions regarding the relative causality of those phenotypes. The progression of swelling can also be associated with leakage of fluid into the eye and increased inflammation, which can ultimately lead to swelling of the macula, the most sight-threatening stage of NPDR called diabetic macular edema (DME). While not detectable on fundus photographs, several studies showed that DME was also characterized by dramatic loss of neuronal function and increased inflammation, confirming the progression of the non-vascular alterations at this stage of the disease[2,3]. In some cases, it is believed that progression of NPDR yields a severe ischemic state in specific areas of the retina, causing production of various factors leading to growth of new blood vessels in a process called neovascularization, marking the transition to PDR[4,5]. These new vessels grow in a less controlled manner and disturb vision by developing in normally avascular regions, such as the vitreous cavity, often leading to hemorrhage, or the macula, leading to dramatic and persistent vision loss. Lastly, scarring or gliosis associated with retinal neovascularization can cause traction between the vitreous and the retina, which can ultimately lead to detachment of the neural retina from the retinal pigment epithelium, a phenomenon called tractional detachment. When taking place in the macula, this detachment is a major cause of severe vision loss in DR patients.

While the diagnosis and associated grading of DR has been more clearly defined over the years, the molecular mechanisms responsible for the different stages of this disease have been more difficult to characterize. Various studies have been conducted using animal models of diabetes to explore the molecular mechanisms of retinal vascular changes and neurodegeneration central to this disease. In contrast, and despite the limitations of the studies based on animal models, only a small number of studies have been conducted focusing specifically on the human pathology. This review will summarize what is presently known about DR from studies using human donor tissues. We will describe how the use of post-mortem tissues has allowed investigation of the vascular, inflammatory, and neuronal aspects of the disease and how it compares to data collected from animal models. Lastly, this review will briefly discuss how this approach enables a better understanding of the pathological mechanisms associated with the individual stages of DR and will explore both the advantages and limitations of using human donor tissues.

VASCULAR ALTERATIONS OF DR

In order to comprehend how changes in the vascular integrity can lead to DR, it is important to understand the basic properties and function of the neuroretina and its vasculature. Retinal capillaries are composed of endothelial cells located on the basement membrane, which is surrounded by pericytes. Connecting neighboring endothelial cells are tight, gap, and adherens junctions, which are responsible for maintaining the integrity of the blood-retinal barrier (BRB). The BRB tightly regulates the permeability of those vessels and is responsible for allowing nutrients and certain elements to reach neuroretinal cells. Blood flow is in turn mostly controlled by the surrounding pericytes through the regulation of the diameter of the vessels. Chronic conditions, such as diabetes, can lead to the disruption of the retinal vasculature over time, leading to the blockage of vessels, leakage of capillaries, and other vascular complications, ultimately resulting in conditions like DR.

Retinal microvascular cell death

Progressive retinal microvessel obliteration marks one of the most significant effects of DR in its early stages, resulting in increased vascular permeability as demonstrated clinically on retinal angiograms. The association between DR and pericyte or endothelial cell death was first described by Mizutani *et al*[6] using trypsin digest preparations and retinal cross-sections from diabetic patients and age-matched non-diabetic controls[6]. Combining TUNEL staining on retinal cross-sections and histological analysis of trypsin digests, the authors first demonstrated an increased number of dying cells in the vascular network of diabetic patients. Morphological analysis coupled with cell-specific immunostaining

demonstrated that both pericytes and endothelial cells were dying in tissues from diabetic patients. To control for structural and functional changes primarily due to aging, rather than diabetes, or even simply post-mortem artifacts, the authors reproduced those findings in two rodent models. The authors reproduced those findings in two rodent models, the alloxan-induced diabetic and galactose-fed rats. This study was one of the first demonstrating the death of endothelial cells and pericytes in retinal tissue from human diabetic patients, and that this phenomenon occurred during the early stages of DR, even before clinical signs could be detected. Subsequently, several studies confirmed those results in independent sets of postmortem human tissues from diabetic patients[7,8]. Detection of a decreased pericyte/endothelial cell ratio was also found, suggesting a higher sensitivity of pericytes compared to endothelial cells that could be associated with their lower capacity to replicate[7].

Neo-angiogenesis

Endothelial and pericyte cell death and the subsequent retinal microvessel degeneration results, among other things, in ischemic foci, which, by way of unmet metabolic needs, can ultimately lead to the initiation of angiogenesis. Previous studies investigating the molecular mechanisms of vascular growth and permeability in DR have identified some of the growth factors involved in angiogenesis in response to diabetes[9]. Non-human primate models involving retinal hypoxia were among the first in which increased mRNA levels of vascular endothelial growth factors (VEGFs) were detected in retina and intraocular fluids after vaso-obliteration[10,11]. Soon after, VEGF was reported to be almost exclusively detected in the intraretinal and choroidal vasculature of diabetic patients[12]. To acquire further insights specific to human vasculature, Mathews *et al*[13] analyzed the retinal vasculature of human post-mortem eyes to determine if a correlation existed between VEGF-positive vessels and vascular permeability. Supporting this hypothesis, the authors observed an increase in the number of VEGF-positive vessels in diabetic eyes compared to non-diabetic eyes. Furthermore, the greatest concentration of VEGF-positive vessels were found in the central retina of diabetic eyes, consistent with prior observations that angiogenesis occurs mainly in this region[14]. Conversely, non-diabetic eyes displayed higher numbers of VEGF-positive vessels within the peripheral retina, results reported in previous studies and likely attributed to degeneration of the eye caused by age or other vascular diseases[15]. Additionally, the authors reported that vascular permeability, assessed by human serum albumin levels, was far greater in diabetic eyes than in non-diabetic eyes. Upon further statistical analysis, the levels of VEGF and human serum albumin in diabetic eyes were directly correlated, whereas no pattern existed in non-diabetic eyes. This represented some of the first evidence that increased permeability of diabetic retinal vessels correlated with increased VEGF levels, even prior to the detection of clinical signs of PDR[13].

While VEGF is a central player in PDR and DME, some evidence points to other factors being involved, including the cytokines tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β or other growth factors such as platelet-derived growth factor[16]. Analysis of vitreous fluid from diabetic patients with PDR showed increased levels of TNF-α[17] which could be part of the pathological mechanism of DR due to its known role in vascular and inflammatory regulation that will be expanded upon later in this review. As for TGF-β, it has previously been shown to have a critical role in the maintenance of the BRB and endothelial cell barrier function[18]. However, one study reported that TGF-β was detected in the photoreceptor layer of both diabetic and non-diabetic human retinal tissue[19], and thus, its exact role in DR remains to be clarified.

Angiogenesis relies not only on different growth factors such as VEGF, TGF-β and TNF-α, but also cell adhesion molecules. The role of cell adhesion molecules is to connect the cell to the external environment as well as neighboring cells by binding proteins that make up the extracellular matrix (ECM) *via* membrane-associated proteins on the cell surface. One class of cell adhesion molecules, integrins, is critical for cell-cell and cell-ECM interaction, thus significantly influencing cellular responses and physiology. Interaction of integrins with the ECM has been shown to influence angiogenesis through regulation of intracellular signaling that affects replication and differentiation of endothelial cells and pericytes. A study conducted by Friedlander *et al*[20] in animal models of angiogenesis suggested that since $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins were involved in angiogenesis, these specific integrins could be critical in PDR. Ning *et al*[21] utilized this data to expand upon and determine the co-localization of five different integrins with the retinal vascular endothelium of four patients with PDR. Contrary to what was found in the animal models, no staining was observed for $\alpha v\beta 5$ integrins, possibly attributed to inter-species variability. As for the other integrins tested, $\alpha 1\beta 1$ and $\alpha 2\beta 1$ integrins were detected in all tissues, at moderate level and without co-localization with endothelial cells. Only $\alpha v\beta 3$ and $\beta 3$ integrin proteins were found to be moderately induced and specifically co-localized with endothelial cell markers in two of the four patients with PDR tested. While further validation is required, these data suggest a specific impact of PDR on a subset of integrins, which could reflect their role in pathological angiogenesis[21].

Perturbations of the retinal vasculature associated with diabetes have long been known from animal models, but studies using tissues from human donors continue to be key to our understanding of the molecular mechanisms underlying the vascular changes associated with the different stages of DR. These studies highlight the complexity of those mechanisms and the interconnection of the vascular pathology with other aspects of the disease

such as the inflammation and neurodegeneration.

INFLAMMATION ASSOCIATED WITH DR

Astrocytes and Müller glial cells, the main macroglial cells of the retina, represent another contributing factor to the diabetes-associated pathology of the neuroretina. In their position between the vasculature and retinal neurons, glial cells play important roles in retinal physiology including regulating permeability of the BRB, supporting neuronal cells, and sensing the extracellular environment, the latter being critical to their regulatory function of retinal inflammation during chronic diseases such as diabetes.

Glial cells dysfunction

Müller cells have long been thought to be affected by diabetes, mostly in their capacity as a support network for the rest of the retina. The first study to investigate the impact of PDR on Müller cells was conducted by Nork *et al*[22] almost 30 years ago, using four post-mortem human eyes. The authors reported the first evidence in human tissues from PDR patients of reactive gliosis demonstrated by the formation of intra-retinal bridges between cystic spaces; and Müller cell dysfunction as suggested by disorganization and migration of their nuclei[22]. More recently, two independent studies sought evidence of abnormalities within Müller cells during the early stages of NPDR. Both studies confirmed reactive gliosis, as demonstrated by increased glial fibrillar acidic protein (GFAP) immunoreactivity in tissues from patients with no to mild NPDR[23,24]. The two studies, however, reported different outcomes regarding the expression of apoptotic regulatory markers, which will be discussed later in this review. GFAP upregulation in diabetic eyes has been demonstrated in various animal models of diabetes and is classically recognized as an indication of cell and tissue reactivity to environmental stress[25,26]. Collectively, these early studies not only confirmed that DR affects Müller cells, but that diabetic conditions promote increased glial cell activation. One of the main functions of glial cells is to regulate the communication between blood vessels and neurons to respond to changes in the environment and maintain retinal homeostasis. Previous studies have suggested that in the early stages of DR, the number of Müller cells increases as astrocyte population decreases, likely as a reaction to the increase in vascular permeability[25,27]. The reported loss of astrocytes in response to diabetes could be enhancing the increased vascular permeability both through mechanical perturbations induced by their absence and loss of the important role they play in the induction of tight junctions within inner retinal blood vessels and the maintenance of the BRB[28,29]. However, the astrocyte loss could also play a pivotal role in the increase in the number of Müller cells, and thus, the enhanced inflammatory response that occurs in the retina in DR.

Inflammatory response

In their original work, Rungger-Brändle *et al*[25] showed that the number of microglial cells, the innate immune cells of the retina, was increased in diabetic rodents. As importantly, they showed that those cells became activated, a state characterized by morphological changes as well as an increase in cytokine production and secretion[25]. While resting microglial cells have multiple, long processes to sense the surrounding environment, activated microglial cells become compact and respond to stress signals by producing various signaling molecules including pro-inflammatory cytokines such as TNF-α, proteases, and reactive oxygen species. There is increasing evidence that inflammation in general, and microglial cell activation in particular, may play an important role in the pathogenesis of DR. One of the premier findings that hinted to this was a study in 1964, when diabetic patients on a salicylate diet, a known anti-inflammatory drug, for treatment of rheumatoid arthritis, reported lower incidence of DR[30]. Subsequent studies displayed similar results, wherein inflammation increased in retinas in response to diabetes; however, most of those have been done in animal models rather than human donor tissues. One such study used streptozotocin-induced diabetic rats to support previously reported findings of an increase in diabetes-associated pro-inflammatory cytokines. Interestingly, and for the first time, this study specifically reported the increase of chemokine (C-C motif) ligand 2 (CCL2) mRNA in the retina of diabetic rats[31]. To this day, data from human retinas remain to be obtained to confirm these observations; however it is supported by human data that were collected by multiple groups from vitreous samples. interleukin-1β and TNF-α, two pro-inflammatory cytokines have been shown to be increased in vitreous samples from patients with DR, even more so in patients with PDR[17]. Several studies also reported CCL2, also known as monocyte chemotactic protein 1 (MCP1), and other cytokines to be elevated in the vitreous of patients with DR[2,32-37]. Muramatsu *et al*[37] also reported that increased levels of cytokines such as MCP-1 correlated with increased VEGF and complement factors levels in the vitreous of PDR patients, while Funatsu *et al*[2] demonstrated an association of cytokine levels with DME, both providing evidence of a link between increased vascular permeability and the inflammatory response in PDR. Of note, analysis of vitreous samples from PDR patients also revealed increased levels of soluble cytokine receptors, a known negative regulatory mechanism of cytokine signaling, suggesting that counter-regulatory mechanisms of angiogenesis and inflammation exist within the eye[38].

In addition to these inflammatory mediators, enhanced expression of intracellular adhesion molecule-1 (ICAM) and P-selectin has been linked to the progression of

DR[39]. ICAM is believed to play a critical role in leukocyte adhesion, one of the initial steps of the inflammatory response allowing leukocytes to cross the BRB in response to increased stress signals. Increased expression of these molecules has been shown to promote the release of inflammatory cytokines, which interfere with endothelial cell tight junction integrity, and thus, increase vascular permeability[40]. While not clearly established, this could be linked with the basement membrane thickening, known to be associated with increased permeability and changes in ECM content in DR patients[41].

As suggested by the observation made by Powell *et al*[30] in 1964, for diabetes as for a wide range of chronic pathologies, a proper control of inflammation is critical to maintaining cellular and tissue homeostasis. While inflammation is a protective mechanism of most complex organisms in response to injury or infectious disease, an uncontrolled inflammatory response becomes part of the pathological mechanism in a variety of chronic diseases such as multiple sclerosis and diabetes. In a study conducted by Krady *et al*[42], the anti-inflammatory drug, minocycline, was suggested to have therapeutic potential in treating/preventing the progression of DR. The authors showed that minocycline treatment leads to a decrease in diabetes-induced cytokine production and reduces microglial cell activation in a rodent model of diabetes. These results were the first to suggest that regulating the inflammatory response could be an important strategy for DR treatment, and that minocycline may be a viable drug to prevent the advancement of DR[42]. More recently, a proof of concept study reported that minocycline oral administration had been associated with improved visual function and regression of central macular edema and vascular leakage in diabetic patients[43] providing further evidence that regulating the inflammatory response can be beneficial to preventing irreversible vascular and neuronal degradation over time.

Overall, the data collected from tissues from human donors along with the data from animal models of diabetes strongly support a role of inflammation in the progression of DR. Seminal work has now been performed that suggests that anti-inflammatory drugs could represent a key component of future therapies for the treatment of DR in order to protect retinal function from the adverse effects of diabetes-associated conditions.

NEURODEGENERATION IN DR

While DR has generally been considered a micro-vascular complication of diabetes, degeneration of the neuroretina has been known for over 50 years. The primary histological analysis of retinal tissue from patients with DR resulted in reports characterizing the loss of cell bodies in every nuclear layers of the retina suggesting a significant loss of retinal neurons in response to diabetes[44,45].

Neuronal cell death

Ganglion cell atrophy and inner nuclear layer (INL) degeneration in retinal tissues from diabetic patients was first described in 1961 and was suggested to happen even prior to vascular changes[46,47]. Soon after, the degeneration of the INL and ganglion cell layers was confirmed in a study using 295 postmortem human eyes and documented the fragmentation of ganglion cell nuclei, which is a characteristic of cells undergoing apoptosis[45]. Following these seminal reports, numerous studies only focused on understanding the vascular aspect of the disease, and additional investigation regarding the neurodegenerative mechanisms only occurred recently. The nature of neuronal cell dysfunction and death associated with diabetes began to be better characterized in a study by Barber *et al*[48] when they reported reduced thickness of the retinal inner nuclear and plexiform layers in 7.5 mo diabetic rats. Retinal ganglion cell (RGC) survival was also decreased by 10% compared to non-diabetic rats. The authors discovered that apoptosis began soon after the induction of diabetes and that the population of cells undergoing apoptosis potentially included ganglion and photoreceptor neuronal cells[48]. More recent findings using rodent models of diabetes have confirmed the diabetes-associated alteration of RGC function and survival (reviewed in[49]). These findings are interesting with regards to reports analyzing flat-mounts and cross-sections of human retinas by immuno-based assays. Consistent with the first report of apoptotic cells in retinas from diabetic human donors distinct from vascular lesions[48], expression of the pro-apoptotic protein Bax was shown to be increased in RGCs of diabetic patients[50]. This study supported the previous findings wherein the progression of diabetes paralleled increased levels of the pro-apoptotic protein Bax in diabetic human retinas, specifically in the inner retina and concentrated in ganglion cells[51]. A subsequent study revealed an increase in levels of Bax, cleaved caspase-3, and caspase-9 in RGCs of diabetic patients supporting the hypothesis that RGCs specifically are undergoing apoptosis during diabetes and providing evidence of neurodegeneration during the early stages of DR[52]. This activation of the apoptotic pathway could be associated with a loss of trophic factors. While the exact impact of diabetes on local insulin signaling in the human retina remains to be fully characterized, rodent models strongly suggest that it is affected as demonstrated by significant reduction of kinase activity of the whole signaling cascade - insulin receptor (IR), insulin receptor substrate 2, phosphoinositide 3-kinase, Akt, and mechanistic target of rapamycin[53,54]. While this could be directly due to loss of insulin signaling, it could also be a result of loss of activation by insulin-like growth factors (IGF). Retinal IR is known to be a spliced variant that is equally susceptible to insulin and IGFs, and IGF1 levels of expression was shown to be decreased in retina from rodent models and human diabetic patients[51]. These

data support a diabetes-associated decrease of trophic factors and subsequent molecular signaling that could lead to retinal cell death.

Apoptosis regulation

In addition to their role in the regulation of inflammation, glial cells and more specifically Müller cells, are involved in supporting neuronal cells, including the regulation of their survival. Regulation of the intrinsic apoptotic pathway is dependent upon expression of members of the anti-apoptotic Bcl-2 protein family. Two independent groups reported Bcl-2 expression in the human retina to be confined to Müller cells; however, one study by Mizutani *et al*[23] reported no change in the expression of the anti-apoptotic protein while the second study by Abu-El-Asrar *et al*[24] reported a small but significant induction of Bcl-2 in samples from diabetic patients compared to age-matched non-diabetic patients. The authors of the second study also reported changes in expression of other anti-apoptotic proteins, which suggests activation of survival pathways, but also increased expression of the cytotoxic effector Fas ligand, which reflects the ambiguous and complex role of Muller glial cells in the molecular mechanisms leading to neuronal cell death[24].

Neurodegeneration is a cumulative process resulting from the complex interplay of several independent stress mechanisms, one of which is oxidative stress. In animal models of diabetes, various cellular stresses have been shown to alter the oxidative state of retinal cells and lead to accumulation of reactive oxygen species (ROS), promoting damages to the cell machinery and ultimately leading to increased cell death. Several studies have reported increased superoxide production, a known marker of oxidative stress, in diabetic rats[55,56]. One of these reported further increased production of superoxide in hypertensive rats in response to diabetes. Additionally, this study showed that those animals presented with increased levels of gliosis and neuronal apoptosis suggesting a link between superoxide production and neurodegeneration in an animal model of diabetes, especially under hypertensive conditions[56]. Despite the growing number of reports suggesting a direct role for oxidative stress in promoting retinal cell death in animal models of diabetes, evidence of such a link between increased oxidative stress and neurodegeneration associated with DR has not been directly shown in human tissues. Indeed, the only data collected so far and supporting this hypothesis come from indirect measures of oxidative stress *via* analysis of the levels of ROS in the retinas of diabetic human donors[57,58]. It is interesting to note that an anterior study using retina samples from twelve human donors with varying durations of diabetes, showed that, within the retina, rod photoreceptors are the most vulnerable to oxidative stress, likely attributable to the high concentration of polyunsaturated fatty acids within their membranes[59]. High lipid concentration, specifically low-density lipoprotein, was shown to be associated with the progression of DR, and recent studies theorize that the retina is highly susceptible to oxidative stress due to its composition of polyunsaturated fatty acids and high oxygen usage[60,61].

Altogether these data strongly support that retinal neurons are highly affected by diabetes and that neurodegeneration is a key aspect of diabetic retinopathy. While it suggests that oxidative stress and loss of trophic factors could play an important role in the induction of apoptosis, it also clearly shows how little we still know about this aspect of the disease and the need for additional studies.

ADVANTAGES AND LIMITATIONS OF POST-MORTEM TISSUES

Advantages

The primary advantage to using human donor samples is that the data collected are directly representative of the disease pathology as opposed to mechanisms uncovered in artificially-induced or genetically-modified animal models of the disease. In addition, the retinas from the majority of the animal models of diabetes do not have the same structural and cellular properties as the human retina. For example, rabbit and guinea pig models are more similar to human in regards to the type and repartition of photoreceptors, but lack an intraretinal vascular network, as opposed to rodent models that possess this vascular network but have a very different rod/cone repartition and properties. Additionally, neither of those models has a macula and thus does not allow investigation of the specific impact of diabetes on this central and key region of the human retina. Another reason of the limited success in translating data obtained from animal models is the heterogeneous nature of the human pathology[62]. In addition to the human-specific variable environmental conditions, none of the animal models routinely used recapitulate the anatomical and regional specificity of the human retina and how it is impacted by diabetes; *i.e.*, peripheral vascular hypoperfusion, non-homogenous visual field impairment, local hemorrhage and lipid exudates, and macular edema.

Limitations

However, it is also important to note the limitations when working with post-mortem human tissues. The first and main concern is the difficulty in promptly processing fresh tissues from human donors compared to animal models, where no consent is needed, and experiments can be planned ahead. Moreover, there is less control over inter-sample experimental variability due to the very diverse background and health history of human donors. In contrast, studies using animal models can be tightly controlled by using inbred strains that have identical

genetic background and are maintained in identical experimental conditions.

CONCLUSION

The goal of this review was to produce an overview of the current state of knowledge regarding the human specificity of the pathophysiology and molecular mechanisms of DR. This review summarizes what has been discovered regarding the impact of diabetes on the vascular, inflammatory, and neuronal components of human retinal tissue. Overall, this review demonstrated that while animal model-based studies can be utilized to address a variety of disease-related questions, studies using human donor tissues are necessary to validate the conclusions from animal models, as well as characterize different molecular mechanisms associated with the individual stages of DR pathology. It also demonstrates the importance for continuous evaluation of the various disease models to assess their efficacy and limitations for investigating specific pathological mechanisms. Finally, this review underscores the gaps in our knowledge concerning even the basic mechanisms regulating vascular alteration, retinal cell survival, and the interplay of various components of the retina in response to diabetes that underline the progression of DR.

REFERENCES

1 **Jackson GR**, Scott IU, Quillen DA, Walter LE, Gardner TW. Inner retinal visual dysfunction is a sensitive marker of non-proliferative diabetic retinopathy. *Br J Ophthalmol* 2012; **96**: 699-703 [PMID: 22174096 DOI: 10.1136/bjophthalmol-2011-300467]

2 **Funatsu H**, Noma H, Mimura T, Eguchi S, Hori S. Association of vitreous inflammatory factors with diabetic macular edema. *Ophthalmology* 2009; **116**: 73-79 [PMID: 19118698 DOI: 10.1016/j.ophtha.2008.09.037]

3 **Greenstein VC**, Chen H, Hood DC, Holopigian K, Seiple W, Carr RE. Retinal function in diabetic macular edema after focal laser photocoagulation. *Invest Ophthalmol Vis Sci* 2000; **41**: 3655-3664 [PMID: 11006264]

4 **Bresnick GH**, De Venecia G, Myers FL, Harris JA, Davis MD. Retinal ischemia in diabetic retinopathy. *Arch Ophthalmol* 1975; **93**: 1300-1310 [PMID: 1200895 DOI: 10.1001/archopht.1975.01010020934002]

5 **Valsania P**, Warram JH, Rand LI, Krolewski AS. Different determinants of neovascularization on the optic disc and on the retina in patients with severe nonproliferative diabetic retinopathy. *Arch Ophthalmol* 1993; **111**: 202-206 [PMID: 7679270 DOI: 10.1001/archopht.1993.01090020056023]

6 **Mizutani M**, Kern TS, Lorenzi M. Accelerated death of retinal microvascular cells in human and experimental diabetic retinopathy. *J Clin Invest* 1996; **97**: 2883-2890 [PMID: 8675702 DOI: 10.1172/JCI118746]

7 **Li W**, Yanoff M, Liu X, Ye X. Retinal capillary pericyte apoptosis in early human diabetic retinopathy. *Chin Med J* (Engl) 1997; **110**: 659-663 [PMID: 9642318]

8 **Boeri D**, Maiello M, Lorenzi M. Increased prevalence of microthromboses in retinal capillaries of diabetic individuals. *Diabetes* 2001; **50**: 1432-1439 [PMID: 11375345 DOI: 10.2337/diabetes.50.6.1432]

9 **Caldwell RB**, Bartoli M, Behzadian MA, El-Remessy AE, Al-Shabrawey M, Platt DH, Liou GI, Caldwell RW. Vascular endothelial growth factor and diabetic retinopathy: role of oxidative stress. *Curr Drug Targets* 2005; **6**: 511-524 [PMID: 16026270 DOI: 10.2174/1389450054021981]

10 **Adamis AP**, Shima DT, Tolentino MJ, Gragoudas ES, Ferrara N, Folkman J, D'Amore PA, Miller JW. Inhibition of vascular endothelial growth factor prevents retinal ischemia-associated iris neovascularization in a nonhuman primate. *Arch Ophthalmol* 1996; **114**: 66-71 [PMID: 8540853 DOI: 10.1001/archopht.1996.01100130062010]

11 **Miller JW**, Adamis AP, Shima DT, D'Amore PA, Moulton RS, O'Reilly MS, Folkman J, Dvorak HF, Brown LF, Berse B. Vascular endothelial growth factor/vascular permeability factor is temporally and spatially correlated with ocular angiogenesis in a primate model. *Am J Pathol* 1994; **145**: 574-584 [PMID: 7521577]

12 **Lutty GA**, McLeod DS, Merges C, Diggs A, Plouét J. Localization of vascular endothelial growth factor in human retina and choroid. *Arch Ophthalmol* 1996; **114**: 971-977 [PMID: 8694733 DOI: 10.1001/archopht.1996.01100140179011]

13 **Mathews MK**, Merges C, McLeod DS, Lutty GA. Vascular endothelial growth factor and vascular permeability changes in human diabetic retinopathy. *Invest Ophthalmol Vis Sci* 1997; **38**: 2729-2741 [PMID: 9418725]

14 **Kohner EM**, Oakley NW. Diabetic retinopathy. *Metabolism* 1975; **24**: 1085-1102 [PMID: 1097858 DOI: 10.1016/0026-0495(75)90102-X]

15 **Wolf S**, Arend O, Schulte K, Ittel TH, Reim M. Quantification of retinal capillary density and flow velocity in patients with essential hypertension. *Hypertension* 1994; **23**: 464-467 [PMID: 8144216 DOI: 10.1161/01.HYP.23.4.464]

16 **Freyberger H**, Bröcker M, Yakut H, Hammer J, Effert R, Schifferdecker E, Schatz H, Derwahl M. Increased levels of platelet-derived growth factor in vitreous fluid of patients with proliferative diabetic retinopathy. *Exp Clin Endocrinol Diabetes* 2000; **108**: 106-109 [PMID: 10826517 DOI: 10.1055/s-2000-5803]

17 **Demircan N**, Safran BG, Soylu M, Ozcan AA, Sizmaz S. Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. *Eye* (Lond) 2006; **20**: 1366-1369 [PMID: 16284605 DOI: 10.1038/sj.eye.6702138]

18 **Walshe TE**, Dole VS, Maharaj AS, Patten IS, Wagner DD, D'Amore PA. Inhibition of VEGF or TGF-{beta} signaling activates endothelium and increases leukocyte rolling. *Arterioscler Thromb Vasc Biol* 2009; **29**: 1185-1192 [PMID: 19461051 DOI: 10.1161/ATVBAHA.109.186742]

19 **Lutty G**, Ikeda K, Chandler C, McLeod DS. Immunohistochemical localization of transforming growth factor-beta in human photoreceptors. *Curr Eye Res* 1991; **10**: 61-74 [PMID: 2029849 DOI: 10.3109/02713689109007611]

20 **Friedlander M**, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresh DA. Definition of two angiogenic pathways by distinct alpha v integrins. *Science* 1995; **270**: 1500-1502 [PMID: 7491498 DOI: 10.1126/science.270.5241.1500]

21 **Ning A**, Cui J, Maberley D, Ma P, Matsubara J. Expression of integrins in human proliferative diabetic retinopathy membranes. *Can J Ophthalmol* 2008; **43**: 683-688 [PMID: 19020635 DOI: 10.3129/i08-145]

22 **Nork TM**, Wallow IH, Sramek SJ, Anderson G. Müller's cell involvement in proliferative diabetic retinopathy. *Arch Ophthalmol* 1987; **105**: 1424-1429 [PMID: 3117025 DOI: 10.1001/archopht.1987.01060100126042]

23 **Mizutani M**, Gerhardinger C, Lorenzi M. Müller cell changes in human diabetic retinopathy. *Diabetes* 1998; **47**: 445-449 [PMID: 9519752 DOI: 10.2337/diabetes.47.3.445]

24 **Abu-El-Asrar AM**, Dralands L, Missotten L, Al-Jadaan IA, Geboes K. Expression of apoptosis markers in the retinas of human subjects with diabetes. *Invest Ophthalmol Vis Sci* 2004; **45**: 2760-2766 [PMID: 15277502 DOI: 10.1167/iovs.03-1392]

25 **Rungger-Brändle E**, Dosso AA, Leuenberger PM. Glial reactivity, an early feature of diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2000; **41**: 1971-1980 [PMID: 10845624]

26 **Barber AJ**, Antonetti DA, Gardner TW. Altered expression of retinal occludin and glial fibrillary acidic protein in experimental diabetes. The Penn State Retina Research Group. *Invest Ophthalmol Vis Sci* 2000; **41**: 3561-3568 [PMID: 11006253]

27 **Gardner TW**, Antonetti DA, Barber AJ, LaNoue KF, Levison SW. Diabetic retinopathy: more than meets the eye. *Surv Ophthalmol* 2002; **47** Suppl 2: S253-S262 [PMID: 12507627 DOI: 10.1016/S0039-6257(02)00387-9]

28 **Lee SW**, Kim WJ, Choi YK, Song HS, Son MJ, Gelman IH, Kim YJ, Kim KW. SSeCKS regulates angiogenesis and tight junction formation in blood-brain barrier. *Nat Med* 2003; **9**: 900-906 [PMID: 12808449 DOI: 10.1038/nm889]

29 **Kakinuma Y**, Hama H, Sugiyama F, Yagami K, Goto K, Murakami K, Fukamizu A. Impaired blood-brain barrier function in angiotensinogen-deficient mice. *Nat Med* 1998; **4**: 1078-1080 [PMID: 9734405 DOI: 10.1038/2070]

30 **Powell ED**, Field RA. Diabetic retinopathy and rheumatoid arthritis. *Lancet* 1964; **2**: 17-18 [PMID: 14149196 DOI: 10.1016/S0140-6736(64)90008-X]

31 **Brucklacher RM**, Patel KM, VanGuilder HD, Bixler GV, Barber AJ, Antonetti DA, Lin CM, LaNoue KF, Gardner TW, Bronson SK, Freeman WM. Whole genome assessment of the retinal response to diabetes reveals a progressive neurovascular inflammatory response. *BMC Med Genomics* 2008; **1**: 26 [PMID: 18554398 DOI: 10.1186/1755-8794-1-26]

32 **Elner SG**, Elner VM, Jaffe GJ, Stuart A, Kunkel SL, Strieter RM. Cytokines in proliferative diabetic retinopathy and proliferative vitreoretinopathy. *Curr Eye Res* 1995; **14**: 1045-1053 [PMID: 8585935 DOI: 10.3109/02713689508998529]

33 **Mitamura Y**, Takeuchi S, Matsuda A, Tagawa Y, Mizue Y, Nishihira J. Monocyte chemotactic protein-1 in the vitreous of patients with proliferative diabetic retinopathy. *Ophthalmologica* 2001; **215**: 415-418 [PMID: 11741107 DOI: 10.1159/000050900]

34 **Hernandez Y**, Arora S, Erb-Downward JR, McDonald RA, Toews GB, Huffnagle GB. Distinct roles for IL-4 and IL-10 in regulating T2 immunity during allergic bronchopulmonary mycosis. *J Immunol* 2005; **174**: 1027-1036 [PMID: 15634927 DOI: 10.4049/jimmunol.174.2.1027]

35 **Abu El-Asrar AM**, Struyf S, Kangave D, Geboes K, Van Damme J. Chemokines in proliferative diabetic retinopathy and proliferative vitreoretinopathy. *Eur Cytokine Netw* 2006; **17**: 155-165 [PMID: 17194635]

36 **Wakabayashi Y**, Usui Y, Okunuki Y, Kezuka T, Takeuchi M, Goto H, Iwasaki T. Correlation of vascular endothelial growth factor with chemokines in the vitreous in diabetic retinopathy. *Retina* 2010; **30**: 339-344 [PMID: 20142713 DOI: 10.1097/IAE.0b013e3181bd2f44]

37 **Muramatsu D**, Wakabayashi Y, Usui Y, Okunuki Y, Kezuka T, Goto H. Correlation of complement fragment C5a with inflammatory cytokines in the vitreous of patients with proliferative diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol* 2013; **251**: 15-17 [PMID: 22527328 DOI: 10.1007/s00417-012-2024-6]

38 **Limb GA**, Hollifield RD, Webster L, Charteris DG, Chignell AH. Soluble TNF receptors in vitreoretinal proliferative disease. *Invest Ophthalmol Vis Sci* 2001; **42**: 1586-1591 [PMID: 11381065]

39 **McLeod DS**, Lefer DJ, Merges C, Lutty GA. Enhanced expression of intracellular adhesion molecule-1 and P-selectin in the diabetic human retina and choroid. *Am J Pathol* 1995; **147**: 642-653 [PMID: 7545873]

40 **Capaldo CT**, Nusrat A. Cytokine regulation of tight junctions. *Biochim Biophys Acta* 2009; **1788**: 864-871 [PMID: 18952050 DOI: 10.1016/j.bbamem.2008.08.027]

41 **Roy S**, Maiello M, Lorenzi M. Increased expression of basement membrane collagen in human diabetic retinopathy. *J Clin Invest* 1994; **93**: 438-442 [PMID: 8282817 DOI: 10.1172/JCI116979]

42 **Krady JK**, Basu A, Allen CM, Xu Y, LaNoue KF, Gardner TW, Levison SW. Minocycline reduces proinflammatory cytokine expression, microglial activation, and caspase-3 activation in a rodent model of diabetic retinopathy. *Diabetes* 2005; **54**: 1559-1565 [PMID: 15855346 DOI: 10.2337/diabetes.54.5.1559]

43 **Cukras CA**, Petrou P, Chew EY, Meyerle CB, Wong WT. Oral minocycline for the treatment of diabetic macular edema (DME): results of a phase I/II clinical study. *Invest Ophthalmol Vis Sci* 2012; **53**: 3865-3874 [PMID: 22589436 DOI: 10.1167/iovs.11-9413]

44 **Liss L**, Wolter JR. Human retinal neurons in tissue culture. *Am J Ophthalmol* 1961; **52**: 834-841 [PMID: 14465815 DOI: 10.1016/0002-9394(61)90909-6]

45 **Bloodworth JM**. Diabetic retinopathy. *Diabetes* 1962; **11**: 1-22 [PMID: 13870120]

46 **Wolter JR**. Diabetic capillary microaneurysms of the retina. *Arch Ophthalmol* 1961; **65**: 847-854 [PMID: 13786452 DOI: 10.1016/0002-9394(61)91802-5]

47 **Wolter JR**. Diabetic retinopathy. *Am J Ophthalmol* 1961; **51**: 1123-1141 [PMID: 13786453 DOI: 10.1001/archopht.1961.01840020849020]

48 **Barber AJ**, Lieth E, Khin SA, Antonetti DA, Buchanan AG, Gardner TW. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. *J Clin Invest* 1998; **102**: 783-791 [PMID: 9710447 DOI: 10.1172/JCI2425]

49 **Kern TS**, Barber AJ. Retinal ganglion cells in diabetes. *J Physiol* 2008; **586**: 4401-4408 [PMID: 18565995 DOI: 10.1113/jphysiol.2008.156695]

50 **Antonetti P**, Flitris Y, Flamant G, Hellio H, Gauthier D, Granier B. Degradation products of the process of thermal recovery of copper from lamina scraps in lab-scale fluidized bed reactor. *J Hazard Mater* 2004; **108**: 199-206 [PMID: 15120873 DOI: 10.1016/j.jhazmat.2003.12.013]

51 **Podestà F**, Romeo G, Liu WH, Krajewski S, Reed JC, Gerhardinger C, Lorenzi M. Bax is increased in the retina of diabetic subjects and is associated with pericyte apoptosis in vivo and in vitro. *Am J Pathol* 2000; **156**: 1025-1032 [PMID: 10702418 DOI: 10.1016/S0002-9440(10)64970-X]

52 **Oshitari T**, Yamamoto S, Hata N, Roy S. Mitochondria- and caspase-dependent cell death pathway involved in neuronal degeneration in diabetic retinopathy. *Br J Ophthalmol* 2008; **92**: 552-556 [PMID: 18369072 DOI: 10.1136/bjo.2007.132308]

53 **Reiter LT**, Seagroves TN, Bowers M, Bier E. Expression of the Rho-GEF Pbl/ECT2 is regulated by the UBE3A E3 ubiquitin ligase. *Hum Mol Genet* 2006; **15**: 2825-2835 [PMID: 16905559 DOI: 10.1093/hmg/ddl225]

54 **Tuscher JJ**, Fortress AM, Kim J, Frick KM. Regulation of object recognition and object placement by ovarian sex steroid hormones. *Behav Brain Res* 2014; Epub ahead of print [PMID: 25131507 DOI: 10.1016/j.bbr.2014.08.001]

55 **Kowluru RA**, Abbas SN. Diabetes-induced mitochondrial dysfunction in the retina. *Invest Ophthalmol Vis Sci* 2003; **44**: 5327-5334 [PMID: 14638734 DOI: 10.1167/iovs.03-0353]

56 **Silva KC**, Rosales MA, Biswas SK, Lopes de Faria JB, Lopes de Faria JM. Diabetic retinal neurodegeneration is associated with mitochondrial oxidative stress and is improved by an angiotensin receptor blocker in a model combining hypertension and diabetes. *Diabetes* 2009; **58**: 1382-1390 [PMID: 19289456 DOI: 10.2337/db09-0166]

57 **Arden GB**, Sivaprasad S. Hypoxia and oxidative stress in the causation of diabetic retinopathy. *Curr Diabetes Rev* 2011; **7**: 291-304 [PMID: 21916837 DOI: 10.2174/157339911797415620]

58 **Hernández C**, Simó R. Neuroprotection in diabetic retinopathy. *Curr Diab Rep* 2012; **12**: 329-337 [PMID: 22581259 DOI: 10.1007/s11892-012-0284-5]

59 **Holopigian K**, Greenstein VC, Seiple W, Hood DC, Carr RE. Evidence for photoreceptor changes in patients with diabetic retinopathy. *Invest Ophthalmol Vis Sci* 1997; **38**: 2355-2365 [PMID: 9344359]

60 **Lyons TJ**, Jenkins AJ, Zheng D, Lackland DT, McGee D, Garvey WT, Klein RL. Diabetic retinopathy and serum lipoprotein subclasses in the DCCT/EDIC cohort. *Invest Ophthalmol Vis Sci* 2004; **45**: 910-918 [PMID: 14985310 DOI: 10.1167/iovs.02-0648]

61 **Nowak JZ**. Oxidative stress, polyunsaturated fatty acids-derived

oxidation products and bisretinoids as potential inducers of CNS diseases: focus on age-related macular degeneration. *Pharmacol Rep* 2013; **65**: 288-304 [PMID: 23744414]

62 **McGonigle P**, Ruggeri B. Animal models of human disease: challenges in enabling translation. *Biochem Pharmacol* 2014; **87**: 162-171 [PMID: 23954708 DOI: 10.1016/j.bcp.2013.08.006]

P- Reviewer: Rahimi R **S- Editor**: Tian YL
L- Editor: A **E- Editor**: Liu SQ

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com