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REVIEW ARTICLE

Neuroretinal Apoptosis as a Vascular Dysfunction in Diabetic Patients

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Abstract: ***Background***: Diabetic retinopathy (DR) is an important complication of diabetes and is considered one of the main causes of blindness in moderate-income and highly-developed countries. As it is a major socioeconomic problem, defining all mechanisms that may lead to DR development is of great importance. In the 21st century diabetic lesions occurring in the retina are well known. However what kind of retinal neuronal damage occurs in the course of diabetes remains unclear.

Results: In this manuscript we present the most recent knowledge about suggested mechanisms of diabetic retinopathy, including neuroretinal apoptosis. Getting a deep insight into the role of apoptosis and degeneration of retinal neurons leading to DR will have vital consequences.

Conclusion: The findings of this review confirm that it is very likely that in the nearest future diabetic retinopathy treatment will be based on administration of neuroprotective agents. The implementation of neuroprotective drugs may slow down retinopathy progression, making it possible to avoid the currently used therapeutic procedures, such as laser photocoagulation, intravitreous injections or posterior vitrectomy, which are not only risky for the healthy part of the retina but also relatively expensive.

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Keywords: Diabetes mellitus, neuronal apoptosis, retina.

1. INTRODUCTION

Diabetes is a disease and a major social issue. Epidemiological studies concerning the incidence of type 1 and type 2 diabetes worldwide show a continuous increase in the number of cases [1-4]. It is believed that the number of diabetic patients will double in Europe and worldwide in the nearest future [1-6]. Changes in the retina developing in the course of diabetes are defined as diabetic retinopathy and considered the most threatening eye complication of the disease. Diabetic retinopathy is referred to as the main cause of blindness in industrialized and middle income countries [7]. According to the World Health Organization in the year 2002 as many as 37 million of the world population were affected by complete blindness, including 4.8% of diabetic retinopathy cases [7]. Since it is a major socioeconomic issue, it is important to determine all mechanisms that may lead to its development [4, 8].

2. RETINA AND DIABETIC RETINOPATHY

Diabetic retinopathy has been clinically divided into non-proliferative and proliferative types. The time from a mild stage to advanced changes varies among individuals. It depends on the disease duration, glycaemia, genetic predispositions and treatment. Additionally, high blood pressure, elevated serum lipids and tobacco smoking promote retinopathy [9]. Hyperglycemia, especially chronic long term hyperglycemia, plays a key role in the pathomechanism of DR. Epidemiological studies indicate that tight glucose control for type 1 patients as well as type 2 patients is recessing micro vascular diabetes complication including diabetic retinopathy [10]. On the other hand, although hyperglycemia is the main mechanism for the changes in the pathomechanism of diabetic retinopathy, recent evidence found out that moderate to severe hypo-glycemia, especially in type 1 diabetes, may contribute to development of neuroretinal apoptosis [11].

The retina shows high metabolic activity in comparison with other tissues. Demand for oxygen and nutrients is so high that they are delivered to the retina *via* double vasculature, through the choroid, supplying blood to the outer layers and primary vasculature of the retina which supplies its inner layers [12]. The imbalance between oxygen demand and supply leads to the risk of ischemic injury in the retina. It is believed that ischemia has a crucial role in the pathomechanism of DR.

The outer layer of the retina is more resistant to hypoxia in contrast to the inner layers [13]. The blood-retina barrier plays a protective role for the neural layer of the retina. The internal blood-retina barrier is formed by vascular endothelial cells. The external barrier consists of retinal pigment epithelium. Tight connections between adjacent endothelial cells and epithelial cells are necessary to control the passage of the

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fluid and soluble substances through the blood-retina barrier and to block permeation of toxic particles and plasma components to the retina [13].

3. VASCULAR CHANGES IN DIABETIC RETINOPATHY

Vascular changes in diabetes affect small vessels (microangiopathy), including precapillary arterioles, capillaries and venules. In the vessels, pericytes become lost, basement membrane is thickened, endothelial cells are damaged and proliferated [14, 15]. As a result of these processes, the vascular lumen becomes narrowed and the amount of flowing blood is decreased. This leads to retinal ischemia and hypoxia. The ischemic retina may produce the hypoxia-inducible factor (HIF) that can release vascular endothelial growth factor (VEGF) to vascularize again the hypoxic areas. Then, VEGF binds VEGF receptors on vascular cells of the retina causing angiogenesis. In this way, new vessels appear on the retina and optic nerve disc (proliferative retinopathy) [16,17]. The elevated level of VEGF causes increased vascular permeability and retinal edema [16]. Additionally, in diabetic patients were observed the higher levels of pro-inflammatory cytokines, such as interleukin-6, 7 and 8 in the aqueous humor and vitreous humor, which increase adherence of leukocytes to vascular endothelial cells [18, 19]. It is believed that chronic inflammation in the vascular walls additionally promotes vessel occlusion and enhances retinal hypoxia [20-22].

Wan *et al*. found out that advanced glycation end products (AGEs) have a key role in the development of DR [23]. AGEs exert deleterious effects inducing vascular changes and cause oxidative stress, influencing crucial pro-inflammatory and pro-sclerotic cytokines [24]. In addition, the receptor for AGEs (RAGE) can be expressed in cells of retina causing inflammatory process. In addition, the AGE-RAGE axis has important role in the neurodegeneration and microvascular changes in DR [25].

4. RETINAL DEGENERATION IN DIABETES

The changes occurring in the retina in the course of diabetes have been well known. However, lesions to the retinal neurons have not been yet fully elucidated. The majority of the available studies have focused on the vascular retinal lesions, assuming that they are the cause of altered neuronal function. This may result from the fact that vascular changes are visible in ophthalmoscopy, whereas the neural retina is transparent. Recently, some authors have suggested that the functional neuronal changes and neuronal viability may contribute to the pathogenic mechanisms of diabetic retinopathy that begin soon after diabetes onset [23-28]. These suggestions seem to be confirmed by abnormal results of electrophysiological examination of the retina in diabetic patients, who also show vascular changes in the eye fundus that exacerbate with the disease progression [13, 30-34]. Thus, neurodegenerative lesions and accelerated apoptosis of retinal neurons are likely to precede vascular changes in the fundus of the eye [27-29].

The layer of the nerve fibers is made up of ganglion cell processes that run along the inner retina and converge at the posterior eyeball to form the optic nerve. The inner limiting membrane is composed of Müller cell basements and joins the vitreous humor basement. Round connections between photoreceptors and Müller glial cells permeate almost the whole thickness of the retina [35]. Müller cells coordinate vascular responses to meet the metabolic demand of neurons, metabolites, neurotransmitters, and help to establish the extracellular environments for membrane potentials and electrical activity [22]. It has been proven that these cells possess properties of stem cells and are capable of developing into many various cells of the retina. Scientists managed to transform these cells *in vitro* into all types of retinal neurons [36, 37]. Changes in the ganglion cells of the retina have been found the earliest in diabetic patients [13, 38]. Their loss caused a decreasing thickness of the retinal nerve fibers layer and was observed in rats with streptozotocin (STZ) - induced diabetes and in diabetic patients with minimal DR [13, 39, 40]. The accelerated apoptosis of ganglion cells coexisted with the changes within Müller cells.

Hyperglycemia and inflammatory processes activate glial cells in the inner retina and cause their migration to the subretinal spaces and the release cytokines, thus leading to the death of neurons [13, 22, 41-44]. These activated cells adhere to the vessels and can damage vascular wall [13, 45]. Schellini *et al*., studied the structure and ultrastructure of retinal Müller cells of healthy Wistar rats, rats with untreated diabetes and those with diabetes treated 1 and 12 months after diabetes induction and observed that the nuclei of the Müller cells were changed in shape and had greater density than other nuclei. In diabetic rats, Müller cells had a dispersed chromatin and increased cytoplasmic content of glycogen and lysosomes. These changes were more frequent in the perivascular region and increased with time. In treated diabetic rats the changes were less pronounced than in the untreated group [46].

Additionally, Müller cells in diabetic patients have been found to show overexpression of glial acidic fibrillar protein (GFAP) [13, 47, 48]. The Müller cells produce agents able to modulate blood flow and vascular permeability and have crucial role in the pathomechanism of diabetic micro-angiopathy in the retina [13, 48].

4.1. Mechanisms Involved in the Process of Neuro-degeneration in DR

It is believed that the major mechanisms involved in the process of neuroretinal injury in diabetic retinopathy include oxidative stress, glutamate accumulation, and decreased retinal production of neuroprotective factors [13, 22].

4.1.1. Glutamate

Glutamate is the primary retinal neurotransmitter and its level is increased in the extracellular space, both in the aqueous and vitreous humor. The accumulation of glutamate can lead to hyperactivation of its ionotropic receptors, such as *alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate* (AMPA) and *N-Methyl-D*-aspartic acid (NMDA), that pass calcium ions to the intracellular space of postsynaptic neurons in an uncontrolled way, causing cells death. The glutamate excess in the extracellular space in diabetes is

caused mainly by its reduced absorption by glial cells, a decrease in the activity of glutamine synthetase, and inhibition in the ability of the retina to convert glutamate to ketoglutarate [13, 49-55].

4.1.2. Oxidative Stress

In the cells, glucose is converted *via* a few metabolic pathways (glycolytic, pentose, hexosamine and polyol). In hyperglycemia, increased glucose metabolism in endothelial cells, monocytes and blood platelets is accompanied by the production of free oxygen radicals. Physiologically, the respiratory chain of the mitochondria is the site where reactive oxygen species (ROS) are produced in small amounts. The reactive oxygen and nitrogen species (RONS) released in physiological amounts have a role of mediators and regulators, ensuring normal cell function. However, the effect of the reactive RONS depends largely on their concentration and time of action. For instance, nitric oxide (NO) produced in small amounts by constitutive NO synthases in endothelial cells of blood vessels plays a major role of a signaling molecule engaged in the regulation of vascular wall tension. However, NO produced in macrophages in high concentrations by inducible synthase shows destructive properties against other cells [56]. The production of free oxygen and nitrogen radicals is under a strict control of the enzymatic and non-enzymatic antioxidant system. If this system fails and/or there is overproduction of RONS, the balance between pro-and anti-oxidative processes can be impaired, which is defined as oxidative stress [57, 58]. Damage induced by RONS within mtDNA is the source of mitochondrial mutagenesis. Abnormal production of the mitochondria, being responsible for the generation of the majority of endogenous ROS, exerts a negative effect on the surrounding structures and on the mitochondria themselves. This leads to a drop in energy production below the required level needed for tissue functioning, and in consequence to functional impairment and premature death also of retinal neurons [59]. In order to minimize the harmful effects of ROS, defensive antioxidant mechanisms are triggered. The most important antioxidants are: glutathione (GSH), vitamins A, C, and E, and the enzymes – glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GRx). The retina is the only neural tissue that is directly exposed to light, leading to the photo-oxidation of lipids. These lipids are toxic to the cells of the retina [13, 60].

Oxidative stress may damage retinal neuronal and microvascular cells. Then, the activity of L-glutamate/L-aspartate transporter (GLAST) is decreased and the accumulation of glutamate increases through Ca2+ channels and ischemia. These results reveal connection between oxidative stress, retinal microvascular changes and neurodegeneration [13].

4.1.3. Neuroprotective Factors

The production by the retina of the neuroprotective factors such as somatostatin (SST), pigment epithelial-derived factor (PEDF) and interstitial retinol-binding protein (IRBP) is lower in patients with diabetes. Decreased expression of the neuroprotective factors compromises neuroprotection against neurotoxic factors connected with neurodegeneration. PEDF is in the main produced by the retinal pigment epithelium (RPE). PEDF inhibits angiogenesis and neurodegeneration in diabetes [61] by reducing oxidative stress in the retina [60, 62] or by increasing the expression of glutamine synthase and the protection against glutamate excitotoxicity [63]. PEDF peptide eye drops have been recently observed to decrease microglial activation, ganglion cell death in diabetic rats, and suggest that exogenous PEDF may be a potential option for treatment early stage of DR [22, 64]. Additionally, PEDF and SST play an important role in homeostasis of the retina because of its anti-angiogenicity and neuroprotection. PEDF inhibits oxidative stress and glutamate toxicity. On the other hand, PEDF downregulation mediates the early vascular changes and favours neurodegeneration. At early DR a lower retinal production of SST and neurodegeneration were observed [13].

VEGF has also neuroprotective properties. Apart from downregulation of natural neuroprotective factors produced by the retina, an upregulation also occurs. In the diabetic retina increased levels of neurotrophic and survival factors such as VEGF and erythropoietin (Epo) were detected, especially in the early stages of DR where ischemia is not the most important event [13].

5. CONCLUSION

The relationship between the toxicity processes involving glutamate and VEGF-induced damage to the blood-retina barrier makes a crucial aspect relating neurodegeneration with vascular disorders. Hyperglycemia is known to induce an increased level in glutamate and then hyperactivity of NMDA receptors, which accelerates death of neurons. It is likely that the hypoxic retina, in order to spare the neurons, initiates the production of the HIF that stimulates the release of VEGF. This factor as a neuroprotector aims to protect the nerve cell through angiogenesis initiation, but increasing vascular permeability damages the blood-retina barrier. Not without significance is the role of the glial cells whose enhanced activity in early stages of diabetes may contribute to nerve cell injury. Additionally, a decrease in the neuroprotective factors, including PEDF and SST may damage the blood-retina barrier through or directly VEGF overexpression.

At the current stage of knowledge is important to thoroughly elucidate the mechanisms of apoptosis and degeneration of retinal neurons causing diabetic retinopathy. Perhaps the treatment of diabetic changes of the retina could be based largely on neuroprotective agents.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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