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Introduction to pathophysiology of diabetic retinopathy

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ABSTRACT

In recent years a systematic growing incidence of diabetes mellitus is being observed. Diabetic retinopathy – a microvascular complication of diabetes mellitus – is one of the leading causes of blindness among professionally active people. Pathophysiology of diabetic retinopathy remains unclear. Decreased retinal perfusion is one of the first observed changes in diabetic retinopathy. Hypoxia and hyperglycemia contribute to diabetic retinopathy development in multiple biochemical mechanism such as: increased sorbitol pathway, increased nonenzymatic protein glycation, increased activation of diacylglycerol and protein kinase C pathway, increased expression of growth factors such as vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1), haemodynamic changes, accelerated oxidative stress, activation of the renin-angiotensin-aldosterone system, subclinical inflammation with leukostasis. There is strong evidence that retinal neurodegeneration contributes to pathophysiology of diabetic retinopathy. A thorough analysis of these underlying pathogenic mechanisms may be useful for new effective pharmacological therapy discoveries.

KEY WORDS: vascular endothelial growth factor, diabetic retinopathy, neurodegeneration, protein kinase C, leukostasis.

INTRODUCTION

In recent years, there has been a steady global increase in the incidence of diabetes mellitus (DM), and the disease has been recognized as a global epidemic. In 2015, the International Diabetes Federation estimated that over the next 25-year period the number of diabetic patients would rise from 415 million to 642 million in 2040 [1]. According to the National Health Fund data, in 2018 a total of 2.86 million adult Poles had DM [2].

One of the major microvascular complications of DM is diabetic retinopathy (DR). In view of poor glycemic control and increasing insulin resistance, diabetic retinopathy is becoming the main cause of blindness in the professionally active age group [3]. Two types of diabetic retinopathy have been identified: non-proliferative (NPDR) and proliferative (PDR), characterized by retinal neovascularization. The main factor contributing to the progression of retinopathy is disease duration. According to epidemiological studies, within 20 years after the diagnosis of diabetes mellitus, almost all patients with type 1 DM and approximately 80% of patients with type 2 DM showed features suggestive of diabetic retinopathy [4].

The pathophysiology of ocular fundus abnormalities associated with DM is very complex and has not been fully elucidated despite many years of research. Reduced retinal blood flow observed already in the initial stage of the disease, neurodegenerative and inflammatory changes, leukostasis, and vascular occlusion lead to hypoxia and further disease progression [5]. This is a simple way of describing the pathogenesis of changes occurring in diabetic retinopathy.

HYPOXIA AND HYPERGLYCEMIA

One of the first changes seen in patients with diabetic retinopathy is decreased retinal blood flow. Already in the early stage of the disease, disorders of retinal capillary circulation are identified. These abnormalities can be assessed by laser Doppler imaging and video fluorescein angiography [6, 7]. Patients develop retinal hypoperfusion and hypoxia.

Hypoxia and hyperglycemia contribute to the development of diabetic retinopathy via a range of mechanisms including increased activity of the sorbitol pathway, increased non-enzymatic protein glycation, and increased activity of the diacylglycerol and protein kinase C pathways, as well as overproduction of growth factors (mainly vascular endothelial growth factor – VEGF, and insulin-like growth factor – IGF-1), hemodynamic alterations, a rise in oxidative stress, activation of the renin-angiotensin-aldosterone system, and subclinical inflammation along with leukostasis [8].

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SORBITOL PATHWAY

In diabetes, excess glucose undergoes transformation in the two-step sorbitol pathway (Figure 1). In retinal cells, aldose reductase (AR) reduces glucose to sorbitol, which is later converted to fructose by sorbitol dehydrogenase (SDH). Sorbitol is impermeable to cell membranes, so it is accumulated in excess and leads to osmotic cellular damage [9]. Increased oxidation of NAPDH – (nicotinamide adenine dinucleotide) to NADP+ and reduction of NAD+ to NADH result in tissue hypoxia. The condition is referred to as hyperglycemic pseudohypoxia [10].

There have been attempts to use hyperactivity in the sorbitol pathway in the pharmacotherapy of diabetic retinopathy. A study in galactose-fed rats showed that aldose reductase inhibitors (ARIs) reduced the frequency and severity of diabetic retinal lesions [11]. However, the efficacy of sorbinil (one of the ARIs) in the treatment of diabetic retinopathy remains doubtful [12]. It appears that the limited therapeutic effects of this drug may be due to the insufficient inhibition of the sorbitol pathway in human tissues compared to animal tissues [12]. Studies evaluating the potential benefit of ARIs other than sorbinil in preventing and reducing the progression of diabetic retinopathy are ongoing, and the results will be known in the near future.

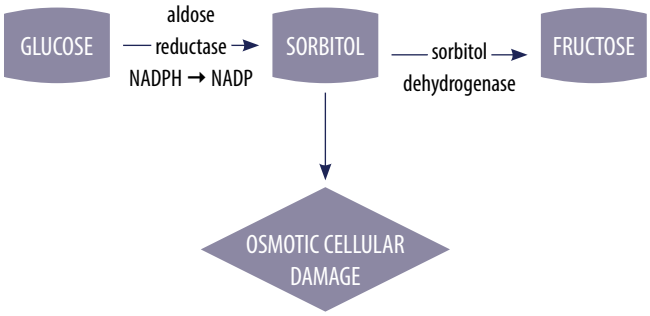

Figure 1. Sorbitol pathway

NON-ENZYMATIC GLYCATION OF PROTEINS

In the human body, the amino residues of proteins, lipids, and nucleic acids react with the aldehyde group of glucose, i.e. non-enzymatic glycation. Under normal conditions, the process occurs at a slow pace and advanced glycation end-products (AGEs) accumulate over time. In patients with diabetes, because of excess glucose availability, the production of AGEs increases, which leads to their excessive accumulation. An excess in the production of AGEs contributes to the development of diabetic retinopathy via several mechanisms. One of them is the accumulation of AGEs intermediates in the matrix, basement membrane, and blood vessel walls [8]. Another mechanism is based on the interaction between AGEs and receptors on the cell membrane walls, e.g. the receptor for advanced glycation end-products (RAGEs), galectin-3 receptor, CD36 and scavenger receptors, which contributes to the activation of inflammation [13, 14]. The resulting AGE-receptor complexes lead to cell dysfunction. The AGE-RAGE complex on the membrane of endothelial cells stimulates their proliferation, increases permeability, and plays a role in blood clot formation [15-17].

Multiple clinical trials have been conducted to evaluate the efficacy of non-enzymatic glycation inhibitors in the treatment of diabetic retinopathy. Treatment with aminoguanidine hydrochloride, an inhibitor of AGE production, was found to reduce pathologies in the blood vessels (damage to pericytes, capillary acellularity, or the formation of microaneurysms) [13].

DIACYLGLYCEROL (DAG) AND PROTEIN KINASE C (PKC) PATHWAYS

Diacylglycerol (DAG) and protein kinase C (PKC) pathways also play a key role in the pathogenesis of diabetic retinopathy. In diabetes mellitus, when glucose is in excess, diacylglycerol (DAG) is synthesized from the intermediates of glycolysis: dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate [18]. An excess of DAG in diabetic patients leads to an increase in the activity of protein kinase C (PKC). PKC is involved in many physiological processes. Increased activity of protein kinase C is a contributor to the development of diabetic retinopathy due to factors including elevated production of extracellular matrix proteins, increased permeability and proliferation of endothelial cells, contraction of smooth muscle cells, leukostasis, VEGF activation, and impaired retinal blood flow [8].

The efficacy of ruboxystaurin, a well-tolerated protein kinase C (PKC) inhibitor, has been evaluated both in experimental and clinical studies. The drug has been proven to be of no benefit in the prevention of diabetic retinopathy [19]. However, clinical studies show that in symptomatic patients (especially those with diabetic macular edema), ruboxyturin may significantly improve visual acuity and reduce the need for laser photocoagulation of the retina [19, 20].

GROWTH FACTORS

Another important mechanism leading to the development of diabetic retinopathy is an increase in growth factor activity. In the context of its effect on the development of retinopathy, the best studied growth factor is VEGF. As mentioned above, VEGF activation occurs as a result of the effects produced by PKC. VEGF plays a role in the breakdown of the blood-retinal barrier, increases vascular permeability, and stimulates the proliferation of vascular endothelial cells and neovascularization [21-23]. In these mechanisms, it induces enhanced retinal angiogenesis. In Poland, intravitreal injections of antineovascular drugs (aflibercept, bevacizumab, ranibizumab) are widely used in the treatment of complications of diabetic retinopathy. Unfortunately, it has increasingly been reported that these substances produce neurodegenerative effects. A study conducted by Hombrebueno found that long-term anti-VEGF administration in diabetic mice caused damage to photoreceptors, amacrine cells, retinal ganglion cells, and synaptic connections [24].

The pathogenesis of diabetic retinopathy also involves other growth factors including the fibroblast growth factor

(FGF), insulin-like growth factor-1 (IGF-1), and platelet-derived growth factor (PDGF) [8]. The mechanism underlying the effect of the growth factors enumerated above on the development of diabetic retinopathy is not fully understood, so their inhibitors are not used in the treatment of diabetic complications.

Hemodynamic alterations manifested as arterial hypertension, as well as the activation of the renin-angiotensin-aldosterone system, are known to contribute significantly to the development of diabetic retinopathy. High blood pressure causes damage to vascular endothelial cells and increases the activity of the renin-angiotensin-aldosterone system [8].

RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

The activity of the renin-angiotensin-aldosterone system (RAAS) – and particularly renin, angiotensin-converting enzymes ACE1 and ACE2, and receptors for angiotensin – has been shown to be increased in proliferative diabetic retinopathy [25, 26]. The mechanism by which the RAAS affects the development of diabetic retinal lesions is uncertain. There are literature reports describing an interaction of angiotensin II with protein kinase C (PKC) and VEGF [27]. An experimental study in mice [28] found a link between Ang1-7, a degradation product of angiotensin II, and diabetic retinopathy [28]. Ang 1-7 inhibits retinal protein O-GlcNAcylation, thus reducing the risk of developing diabetic fundus lesions [2]. The application of this mechanism in the treatment of DR may become an alternative to ACE-inhibitors and angiotensin receptor blockers (ARBs) (Figure 2) [33].

LEUKOSTASIS

As early as a few weeks after the onset of diabetes, a phenomenon referred to as leukostasis is known to occur. Leukocytes adhere closely to the vascular endothelial cells, which leads to the occlusion of small vessels, reduced blood flow, and local ischemia [29]. Diabetes mellitus is associated with elevated levels of pro-inflammatory cytokines and adhesion molecules, which attracts leukocytes and affects the reactions of leukocytes with endothelial cells [29]. Studies have shown that the levels of leukocytes, pro-inflammatory cytokines and adhesion factors are correlated with the degree of progression of diabetic retinopathy [31].

Attempts have been undertaken to use the anti-inflammatory mechanism in the pharmacotherapy of diabetic retinopathy. Intravitreal injections of steroids (triamcinolone) or non-steroidal anti-inflammatory drugs (nepafenac) are effective in reducing the symptoms of diabetic retinopathy, especially in its advanced stages. However, they carry the risk of side effects which are typically associated with intravitreal injections, e.g. glaucoma or intraocular inflammation [31]. Eye drops are recognized as the most desirable drug dosage form, resulting in the least complications or threats to visual function. In Poland, a commonly used drug in the form of eye drops is nepafenac, characterized by high efficacy in the treatment of diabetic macular edema [34].

OXIDATIVE STRESS

Another mechanism implicated in the pathogenesis of diabetic retinopathy is oxidative stress. It is known to play a key role in the pathophysiology of diabetes mellitus: under conditions of hyperglycemia and oxidative stress, increased glycolysis, NADP overproduction, and increased generation of free oxygen radicals (ROS) occur [10]. In experimental studies in diabetic rats, an elevated level of free radicals in retinal cells was detected, as compared to the control group (rats with normal blood glucose levels) [32]. Reactive oxygen species lead to cell apoptosis, including the apoptosis of endothelial cells, which results in increased leukostasis. A re-

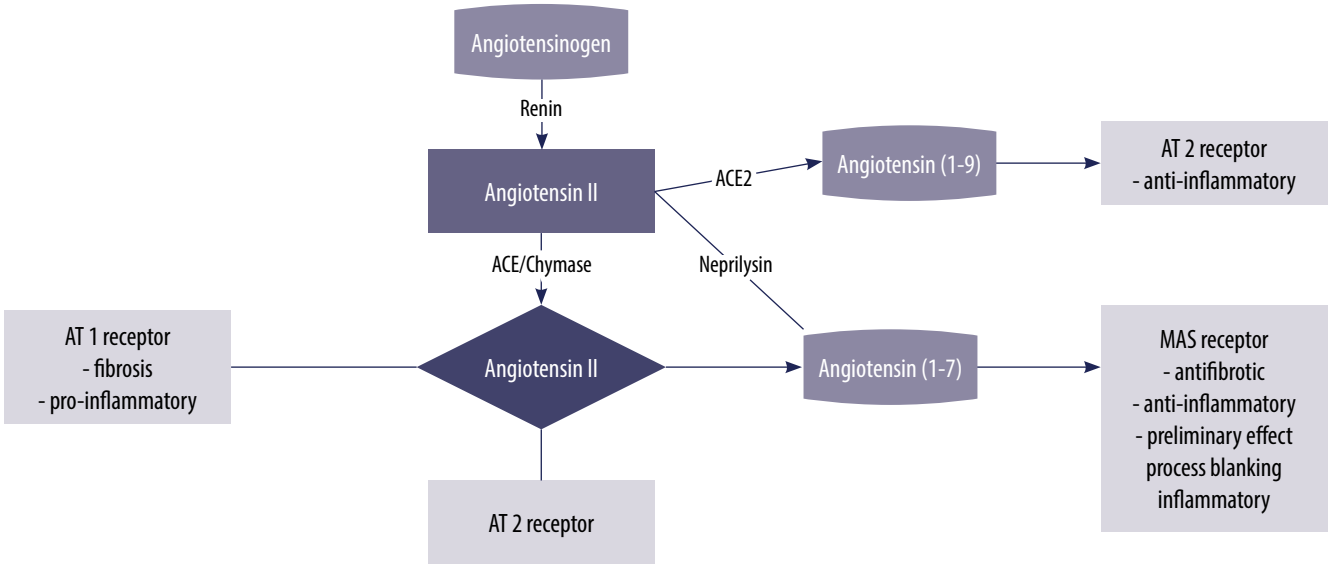

Figure 2. Renin-angiotensin-aldosterone system

duction in oxidative stress levels has also been found to affect other mechanisms playing a significant role in diabetic retinopathy, inhibiting the sorbitol pathway and decreasing protein kinase C activity [10].

NEURODEGENERATIVE CHANGES

The final topic that needs to be addressed in this paper relates to the characteristics of neurodegenerative changes in the pathogenesis of diabetic retinopathy. According to the available studies, neurodegenerative alterations – gliosis, decreased activity and apoptosis of retinal nerve cells – appear before the development of changes associated with diabetic microangiopathy [35-37]. Retinal ganglion cells and amacrine cells are most at risk of "programmed death", but apoptosis also affects the photoreceptors: cones and rods [38].

Neurodegenerative changes in diabetes are evaluated by OCT and mfERG (multifocal electroretinography). OCT shows a reduced thickness of the retinal nerve fibers (RNFL), and mfERG – prolonged wave latency [39].

There are many potential mechanisms of cellular apoptosis in diabetic retinopathy. Those most commonly mentioned include increased extracellular glutamate accumulation, decreased levels of neural cell survival factors (e.g. brain-derived neurotrophic factor (BDNF), insulin, platelet-derived growth factor (PDGF)), increased oxidative stress, and local inflammatory process with leukostasis [40].

Several attempts have been made to apply the neurodegenerative theory in the pharmacotherapy of diabetic retinopathy. A randomized study conducted by Simon *et al.* (2017) failed to provide evidence for the efficacy of neuroprotective drugs (brimonidine and somatostatin) administered topically in the form of eye drops in the prevention and progression of diabetic retinopathy [41]. However, it is conceivable that other studies using the mechanism in the treatment of diabetic complications will be undertaken in the future.

CONCLUSIONS

The paper describes several pathways with a significant role in the pathogenesis of diabetic retinopathy. Most of them are linked to one another, mainly through protein kinase C activity, elevated VEGF activity or leukostasis. Although scientists across the globe have been studying the pathophysiology of diabetic retinopathy for years, exploring mechanisms leading to the development of DR-related complications, there are as yet no effective prevention and treatment methods that would ensure good visual acuity in patients over long-term follow-up. Hopes are being placed on new retinal imaging techniques as a potential path towards the development of an innovative effective therapy for diabetic retinopathy.

DISCLOSURE

The authors declare no conflict of interest.

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