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REVIEW

Antiangiogenic cytokines as potential new therapeutic targets for resveratrol in diabetic retinopathy

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Abstract: Diabetes mellitus (DM) affects >350 million people worldwide. With many complications that can reduce the patient's quality of life, vision loss is one of the most debilitating disorders it can cause. Active research in the field of diabetes includes microvascular complications in diabetic retinopathy (DR). Disturbances in the balance of pro-angiogenesis and anti-angiogenesis factors can lead to the progression of DR. The retinal pigment epithelium (RPE) is the outermost layer of the retina, and it is essential in maintaining the visual function. The RPE produces and secretes growth factors as well as protective agents which maintain structural integrity of the retina. Small natural molecules, such as resveratrol, may influence neurotrophic factors of the retina. The pigment epithelium-derived factor (PEDF) and thrombospondin-1 (TSP-1) are secreted by RPE cells. These two proteins inhibit angiogenesis and inflammation in RPE cells. An alteration of their production contributes to various eye diseases. There is a critical balance between two important factors secreted on opposite sides of the RPE: at the basal side, vascular endothelial growth factor (VEGF; acts on the choroidal endothelium) and, on the apical side, PEDF (acts on neurons and photoreceptors). Resveratrol inhibits VEGF expression in human adult RPE cells and limits the development of proliferative vitreoretinopathy, by attenuating transforming growth factor-β2-induced wound closure and cell migration. Possible new mechanisms could include PEDF and TSP-1 expression alterations under physiological and pathological conditions. Resveratrol is currently of interest due to its capacity to influence the cell's secretory activity. Some limitations arise from its low bioavailability. Several drug delivery systems are currently tested, promising to improve tissue concentrations. This article reviews biological pathways involved in the pathogenesis of DR that could be influenced by resveratrol. A study of these pathways could identify new potential targets for the reduction of diabetic complications.

Keywords: diabetes, retinal secretome, diabetic microvascular complications, phytoalexin

Introduction

Diabetes mellitus (DM) affected >350 million people worldwide in 2013, and the number is estimated to increase to >590 million people by 2035.[1] Without proper treatment, its complications are serious, starting with ketoacidosis, nonketotic hyperosmolar coma, and continuing with serious vessel damage: microangiopathies (retinopathies, neuropathies, or nephropathies) and macroangiopathies (peripheral vascular diseases, ischemic cardiomyopathy, or cerebrovascular accidents). In the long term, these can be an important cause of mortality. One of the leading causes of blindness in adults aged 20–74 years is diabetic retinopathy (DR).[2] In DR, progressive damage to the retina

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occurs when fragile blood vessels break, causing swelling of the retinal tissue and cell loss.

Current treatments are trying to achieve strict glycemic control. A recent European epidemiological study showed that, despite modern protocols, 39% of young adults diagnosed with diabetes in the last 10 years develop DR, and the need for protection from vision loss is greater than ever.[3] In 2012, Yau et al[4] studied the global prevalence and risk factors of DR. The conclusion of the study was an overall prevalence of 34.6% DR, 7% proliferative DR (PDR), 6.8% diabetic macular edema (DME), and 10.2% vision-threatening DR. DR was present in nearly all diabetic patients after a certain period, with a higher prevalence in type 1 diabetes.[4] Once diagnosed, DR was directly linked to insufficient metabolic control; the strongest predicting risk factors for retinopathy are HbA1c and glycated albumin.[5] A study performed in 2010 revealed that approximately one third of 285 million people diagnosed with diabetes showed signs of DR, and the number is expected to rise further.[6]

Standard care for PDR includes panretinal photocoagulation (PRP) and more recently anti-vascular endothelial growth factor (VEGF) injections.[7–9] PRP was initiated as a treatment option in the 1960s and was documented as beneficial over time. It is used to improve the retinal circulation, allowing more oxygen to reach the retina and limiting the formation and release of pro-angiogenic cytokines.[10] Laser treatment can reduce the rates of vision loss by up to 50% over 3 years.[11] Anti-VEGF injections have been recently used to treat PDR and proved to be a promising potential alternative. A multicenter randomized clinical trial comparing ranibizumab versus PRP recently concluded that ranibizumab has similar effects in improving visual acuity at a 2-year follow-up. Ranibizumab proved superiority over PRP when it came to safety concerns. It showed fewer side effects, such as peripheral visual field loss or contrast sensitivity loss. Although intravitreal ranibizumab of 0.5 mg is more expensive than PRP by almost 60%, its reduced adverse effects may support its future use.[7]

DME affects ~30% of patients with DR and is a major factor leading to vision loss.[12] It can occur at any stage of DR, and the risk increases with the severity of the disease: 3% cases with mild nonproliferative diabetic retinopathy (NPDR), 38% cases with moderate-to-severe NPDR, and 71% cases with PDR.[13] Intravitreal VEGF inhibitors are currently the standard care for DME. Following the results of the Ranibizumab for Edema of the mAcula in Diabetes (READ-2) study after 6 months, 2 years, and 3 years, ranibizumab has become a frequently used first-line therapy agent for DME.[14–16] Intravitreal steroid implants (such as dexamethasone or fluocinolone acetonide) exert their anti-inflammatory action, with minimum side effects, and have a nonspecific anti-VEGF effect. For this reason, they are currently reimbursed by different health care systems in the management of DME.[12] There is a lack of consensus on the management of patients with DME who underwent vitrectomy. For this reason, current studies evaluate the outcome of steroid implants, such as fluocinolone acetonide, on visual acuity and patient compliance, suggesting that further research and real-life data collection might support the use in current practice, as well as in earlier DME development stages.[17] Refractory cases of DME to ranibizumab or dexamethasone could be switched to aflibercept. Studies comparing the two agents (ranibizumab and aflibercept) offer controversial results. Evidence suggests that the two agents are equal in terms of efficiency, with aflibercept having the advantage of fewer injection numbers required.[18] One case–control study on a 69-year-old man with bilateral DME showed a significant improvement with aflibercept over ranibizumab treatment. A plausible explanation might be the additional capacity of aflibercept to target the placental growth factor-1, a member of the VEGF family.[19] Given the fact that aflibercept is well tolerated, has a comfortable dosage regimen, and has a complex mechanism of action, it might be considered for DME first-line treatment.[20] Future prospective studies need to focus on a long-term follow-up regarding safety and efficacy when switching from one anti-VEGF agent to another.[21]

With strong level 1 evidence supporting anti-VEGF injections over PRP for the treatment of DME, new data suggest that it may become a key player in the treatment of DR and PDR.[22] Anti-VEGF agents may slow down the development of DME in preproliferative eyes, but data are scarce regarding DR progression after anti-VEGF injections are discontinued. Some important issues need more clarification: cost, prospective studies on other VEGF agents, and new sustained-release devices. While PRP remains the standard care for PDR, combination therapy could be a valid alternative: anti-VEGF initially followed by PRP.[7]

The Early Treatment Diabetic Retinopathy Study classification covers the full range of retinopathies and is the most complex one.[11] In 2002, the Global Diabetic Retinopathy Project Group proposed a version designed to be used for population screening[23] (Table 1).[24]

In the past few years, natural polyphenols, found in plants, including vegetables and fruits, have gained interest, and medical nutrition therapy is emerging as a tailored therapeutic approach that could reduce the risk of developing complications in chronic diseases such as type 2 diabetes.

Table 1 Classification of DR severity

International clinical DR disease severity scale			
Mild NPDR	**Moderate NPDR**	**Severe NPDR**	**PDR**
Microaneurysms only	More than just microaneurysms Less than severe NPDR	>20 intraretinal hemorrhages in each of 4 quadrants OR Definite venous bleeding in 2+ quadrants OR Prominent IRMA in 1+ quadrant and no PDR	One or more: Neovascularization Vitreous/preretinal hemorrhage

Notes: Adapted from: The Royal College of Ophthalmologists. Diabetic retinopathy guidelines; December 2012. Available from: https://www.rcophth.ac.uk/wp-content/uploads/2014/12/2013-SCI-301-FINAL-DR-GUIDELINES-DEC-2012-updated-July-2013.pdf. © The Royal College of Ophthalmologists 2012. All rights reserved.[24]
Abbreviations: DR, diabetic retinopathy; IRMA, intraretinal microvascular abnormalities; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

The search for new substances to diminish vision loss due to DM includes natural products, with resveratrol emerging as a promising "player."[25] This polyphenolic phytoalexin has beneficial properties according to several in vitro and in vivo studies. Such benefits include antidiabetic activity,[26] anti-inflammatory effect,[27] anti-neovascularization protection,[28] and prevention of DR.[29]

Biochemical mechanisms involved in DR

Diabetes, in all its forms, is characterized mainly by hyperglycemia. During its course, the development of microvascular pathology is likely. Figure 1 shows the four main mechanisms of hyperglycemia-induced damage that are considered responsible for the occurrence of DR: increased polyol pathway flux, increased advanced glycation end-products (AGEs) formation, increased hexosamine pathway flux, and activation of protein kinase C (PKC). Each of these pathways leads to an abnormal function and production of the cells, resulting in early apoptosis, progressive capillary occlusion, extracellular matrix overproduction, and deposition of plasma proteins.[30,31] These pathways to DR are associated with an overproduction of reactive oxygen species (ROS), and they are discussed next.

Increased polyol pathway flux

This two-step metabolic pathway is an important contributor to DR. The key enzyme of the polyol pathway is aldose reductase (AR), a cytosolic, rate-limiting, monomeric oxidoreductase. The second enzyme involved

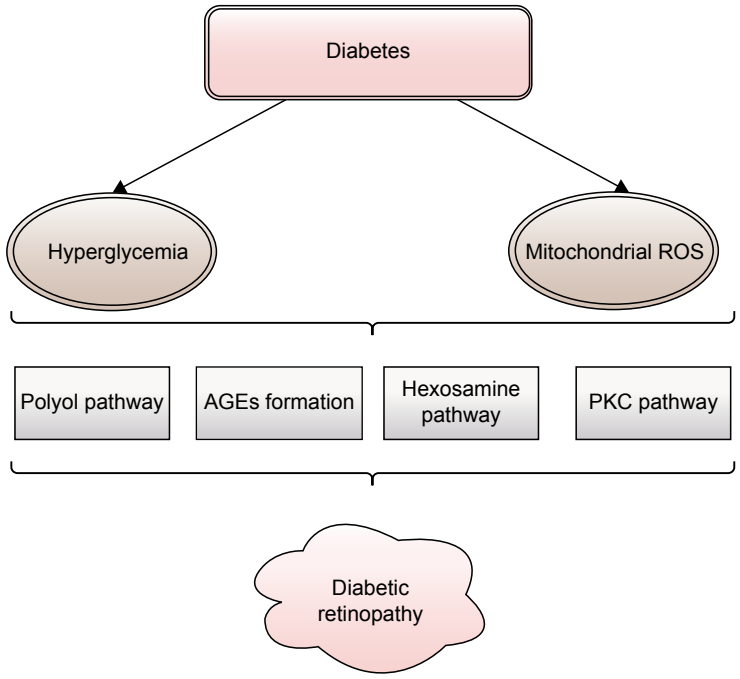

Figure 1 The main mechanisms of hyperglycemia-induced damage considered responsible for the occurrence of diabetic retinopathy: increased polyol pathway flux, increased AGEs formation, increased hexosamine pathway flux, and activation of PKC.
Notes: Adapted from Safi SZ, Qvist R, Kumar S, Batumalaie K, Ismail IS. Molecular mechanisms of diabetic retinopathy, general preventive strategies, and novel therapeutic targets. *Biomed Res Int*. 2014;2014:801269. Copyright © 2014 Sher Zaman Safi et al.[31]
Abbreviations: AGEs, advanced glycation end-products; PKC, protein kinase C; ROS, reactive oxygen species.

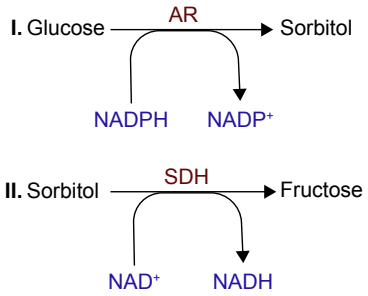

Figure 2 Schematic presentation of stages of polyol pathway: (**I**) glucose reduction to sorbitol by AR (using NADPH as a cofactor) followed by (**II**) sorbitol oxidation to fructose by SDH (using NAD^+ as a cofactor).
Notes: Adapted from Safi SZ, Qvist R, Kumar S, Batumalaie K, Ismail IS. Molecular mechanisms of diabetic retinopathy, general preventive strategies, and novel therapeutic targets. *Biomed Res Int.* 2014;2014:801269. Copyright © 2014 Sher Zaman Safi et al.[31]
Abbreviations: AR, aldose reductase; NAD^+, nicotinamide adenine dinucleotide – oxidized; NADH, nicotinamide adenine dinucleotide – reduced; $NADP^+$, nicotinamide adenine dinucleotide phosphate – oxidized; NADPH, nicotinamide adenine dinucleotide phosphate – reduced; SDH, sorbitol dehydrogenase.

is sorbitol dehydrogenase (SDH). Under euglycemic conditions, AR has a low affinity for glucose, but a high capacity of conversion, reducing it to sorbitol (with nicotinamide adenine dinucleotide phosphate – reduced [NADPH] as a cofactor) at a low level. SDH has a high affinity, but a low capacity to oxidize sorbitol to fructose (using nicotinamide adenine dinucleotide – oxidized [NAD^+] as a cofactor) independent of its concentration within physiological values (Figure 2). In diabetic patients, the polyol pathway activity increases in the retina, causing local accumulation of sorbitol and osmotic damage. Other factors that contribute to further damage are a decrease in cytosolic NADPH and an increase in cytosolic nicotinamide adenine dinucleotide – reduced (NADH)/NAD^+ under hyperglycemic conditions.[31]

The alteration in the NADH (NADPH)/NAD^+ ($NADP^+$) ratios reduces the activity of glutathione reductase, lessening the cell's ability to respond to ROS accumulation, while the increase in NADH/NAD^+ mimics hypoxia in the tissue (Figure 3).[31,32]

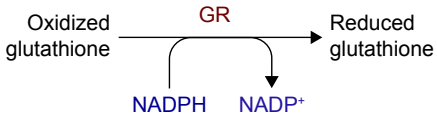

Figure 3 Polyol pathway changes induced in NADH (NADPH)/NAD^+ ($NADP^+$) ratios with the decrease of GR, leading to ROS accumulation and tissue impairments.
Notes: Adapted from Safi SZ, Qvist R, Kumar S, Batumalaie K, Ismail IS. Molecular mechanisms of diabetic retinopathy, general preventive strategies, and novel therapeutic targets. *Biomed Res Int.* 2014;2014:801269. Copyright © 2014 Sher Zaman Safi et al.[31]
Abbreviations: GR, glutathione reductase; NAD^+, nicotinamide adenine dinucleotide – oxidized; NADH, nicotinamide adenine dinucleotide – reduced; $NADP^+$, nicotinamide adenine dinucleotide phosphate – oxidized; NADPH, nicotinamide adenine dinucleotide phosphate – reduced; ROS, reactive oxygen species.

Increased AGEs formation

Prolonged hyperglycemia amplifies certain physiological nonenzymatic processes, resulting in the formation of a complex and irreversible group of compounds termed AGEs.[33] Glucose derivatives enter a condensation reaction (Maillard reaction) with the amine residues of proteins, nucleic acids, or lipids and follow a series of chemical rearrangements, leading to structural and functional degradation that in turn leads to mural pericyte loss and vascular lesions.[33] Markers of AGEs formation and accumulation are found in high concentrations in diabetic patients in retinal vessels, in vitreous tissues, and in other tissues affected by hyperglycemia.[34,35] There are three general mechanisms through which AGEs target cells: modification of intracellular proteins resulting in an altered function, modification of matrix components resulting in an abnormal interaction between each other and with cellular protein receptors (integrins), and modification of plasma proteins, which bind to specific AGEs receptors (receptor for AGE [RAGE]) inducing an increased production of ROS (Figure 4).[30] RAGE ligation activates transcription factor nuclear factor-κB (NF-κB), which in turn induces abnormal gene expression, as well as NADPH oxidase that contributes to ROS formation and pericyte apoptosis.[36–38]

The polyol and the increased AGEs formation pathways intertwine. The fructose produced through the polyol pathway undergoes further transformations, generating glycation agents that can produce AGEs.[39] These factors increase VEGF

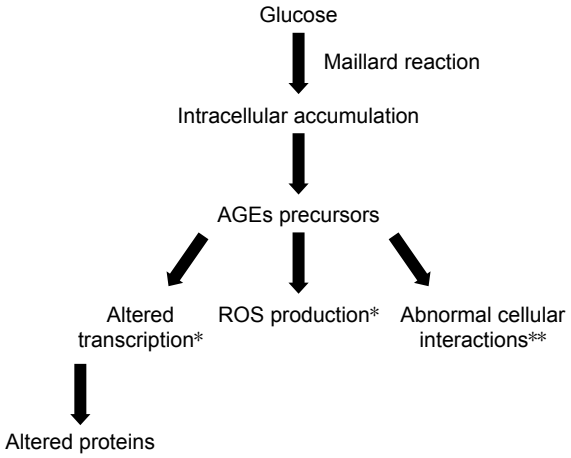

Figure 4 Schematic presentation of AGEs formation and the pathways through which AGEs target cells: altered function of intracellular proteins; abnormal interaction between matrix components and protein receptors (integrins); increased production of ROS due to abnormal interaction between plasma proteins and specific AGE receptors.
Notes: *Intracellular actions; **extracellular actions. Original figure; adapted from data from Brownlee.[30]
Abbreviations: AGEs, advanced glycation end-products; ROS, reactive oxygen species.

gene transcription, mediating capillaries hyperpermeability and intercellular adhesion molecule (ICAM) production and influencing retinal capillary leukocyte adherence.[40]

Increased hexosamine pathway flux

This hyperglycemia-induced pathway impacts the development of diabetic complications. In its glycolytic pathway, glucose is normally converted to glucose-6-phosphate and then isomerized to fructose-6-phosphate (F-6-P). In hyperglycemic conditions, F-6-P is diverted from glycolysis to *N*-acetylglucosamine-6-phosphate by glutamine–fructose-6-phosphate aminotransferase (GFAT). The conversion continues with end-products such as proteoglycans and *O*-linked glycoproteins (cytoplasmic and nuclear proteins).[41] Inhibition of GFAT blocks the increased transcription of transforming growth factor-alpha, transforming growth factor-beta 1 (TGF-β1), inducers of cell proliferation and differentiation, and plasminogen activator inhibitor-1 (PAI-1), involved in cell differentiation, growth, and apoptosis.[31,41] Through the hexosamine pathway, hyperglycemia induces changes in gene expression and protein function, that contribute to the pathogenesis of DR (Figure 5).

Activation of PKC

PKC is a family of multifunctional serine/threonine kinases divided into three groups: classical (PKC-α, PKC-β1, PKC-β2, and PKC-γ), novel (PKC-δ, PKC-ε, PKC-η/l, and PKC-θ), and atypical (PKCζ and PKCλ/ι).[42] Studies showed that intracellular hyperglycemia increases de novo diacylglycerol synthesis, a lipid second messenger that activates the classical PKC isoforms.[43] Hyperglycemia can also activate PKC isoforms through the ligation of AGEs receptors[44] and increase flux through the polyol pathway.[45] Oxidants such as H_2O_2 can activate PKCs through a mechanism unrelated to lipid second messengers[46] through mitochondrial superoxide induced by hyperglycemia.[47] PKC activation alters the bioavailability of nitric oxide, affects VEGF expression (by decreasing the expression of prostacyclin and increasing the expression of thromboxane),[48] and directly increases albumin and other macromolecules permeability through barriers formed by endothelial cells.[49,50] It has also been linked to mitogen-activated protein kinase activation, a factor that increases gene expression (Figure 6).[43,51]

In previous studies, it has been stated that PKC activation is responsible for major changes resulting in diabetes complications: vascular permeability, increased blood flow, extracellular matrix protein accumulation, membrane hypertrophy, leukocyte adhesion, angiogenesis, and apoptosis.[52–54]

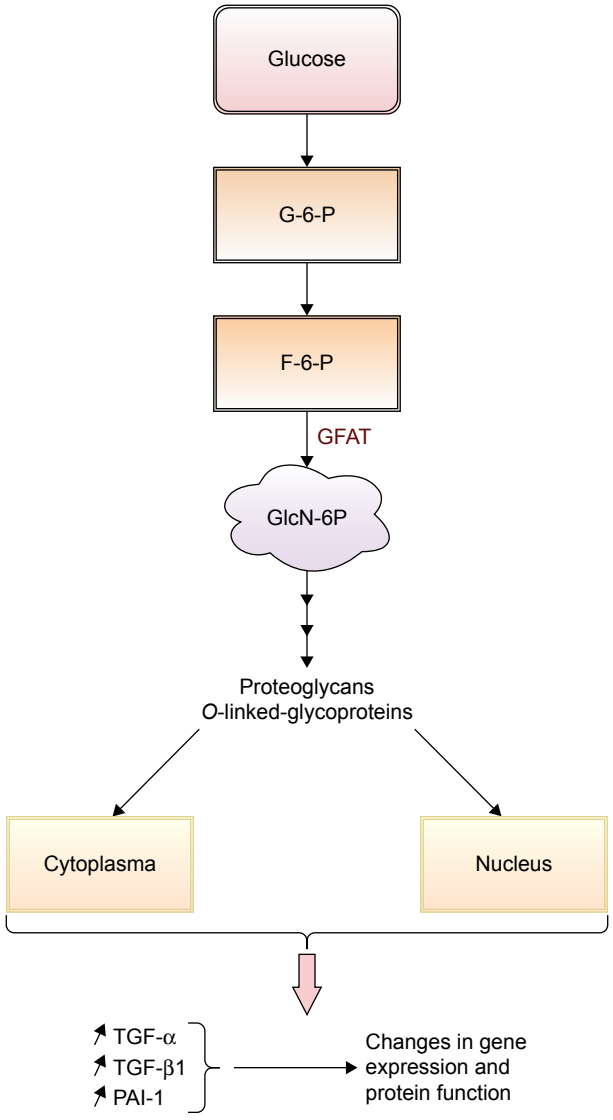

Figure 5 Schematic presentation of the contribution of increased hexosamine pathway flux in pathogenesis of DR.
Note: Original figure; adapted from data from Giacco and Brownlee.[41]
Abbreviations: DR, diabetic retinopathy; F-6-P, fructose-6-phosphate; G-6-P, glucose-6-phosphate; GFAT, glutamine–fructose-6-phosphate aminotransferase; GlcN-6P, N-acetylglucosamine-6-phosphate; PAI-1, plasminogen activator inhibitor-1; TGF-α, transforming growth factor-alpha; TGF-β1, transforming growth factor-beta 1.

Hyperglycemia-induced pathological changes in the retina

These four biochemical pathways linked to an altered glucose metabolism disturb the balance of pro-angiogenesis and antiangiogenesis factors involved in the progression of DR. An increase in the polyol pathway flux correlates with

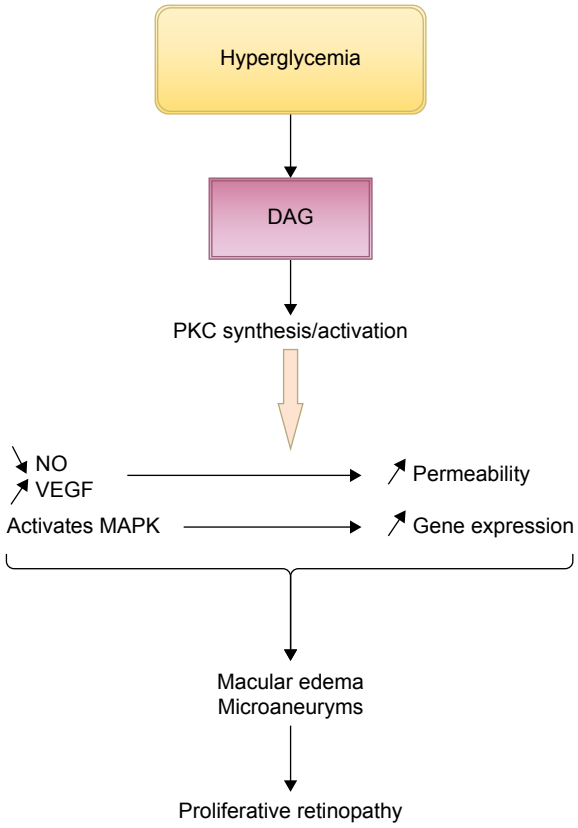

Figure 6 Schematic presentation of the contribution of PKC activation to the development of diabetic retinopathy.
Note: Original figure; adapted from data from Geraldes and King.[43]
Abbreviations: DAG, diacylglycerol; MAPK, mitogen-activated protein kinase; NO, nitric oxide; PKC, protein kinase C; VEGF, vascular endothelial growth factors.

tissue hypoxia, which in turn triggers VEGF production.[55] A recent in vitro study also showed an increase in VEGF-C secretion by AGEs, followed by the increased effect of VEGF-A, underlining the role of AGEs in the onset of retinal neovascularization.[56]

The hexosamine pathway increases TGF-β1 synthesis through the GFAT enzyme. Inhibition of this pathway lowers TGF-β1 synthesis and bioactivity associated with the development of DR.[57,58] Overactivity of PKC also alters VEGF production causing retinal vascular dysfunctions, while in vivo studies showed an association between platelet-derived growth factor (PDGF) resistance and the increase of retinal PKC-δ in diabetic mice. Inhibition of the survival signaling pathway of PDGF causes retinal cell apoptosis.[54]

For this reason, new pharmacological treatments are based on understanding the molecular mechanisms that occur and the factors involved in the process. The retinal pigment epithelium (RPE) is the outermost layer of the retina, and it has several functions, making it essential in assuring our visual function: transporting nutrients and metabolism products through the blood–retinal barrier, light absorption, secretion, and immunity. This monolayer of pigmented cells is well documented in producing and secreting certain types of growth factors, as well as protective agents, which maintain structural integrity. RPE cells secrete proteins at two opposite poles: at the apical cell side or at the basal side.[59] The angiogenic/antiangiogenic factor ratio balances the cellular redox status (or cellular oxidative status), with >55 differentially secreted proteins in hyperglycemic conditions.[60] Table 2 lists some of them.

New potential targets

Research in the field of DR has focused mainly on pro-angiogenic compounds, while protective factors have received less attention. In healthy adults, endogenous antiangiogenic factors, such as thrombospondin-1 (TSP-1), pigment epithelium-derived factor (PEDF), and angiostatin,[61–63]

Table 2 Proteins secreted from RPE cells

Cytokines secreted by RPE	Functions	Role in DR
VEGF	Proinflammatory and angiogenic molecule	Regulates neovascularization
Transforming growth factor-β	Controls proliferation and cellular differentiation	Alters growth factor's balance
Insulin-like growth factor-I	Promotes cellular growth and insulin-like metabolic effects	Autocrine/paracrine regulation of proliferation
Platelet-derived growth factor	Regulates cell growth and division and pericyte viability	Promotes neovascularization and traction of epiretinal membranes
Lens epithelium-derived growth factor	Growth and survival factor	Enhances survival in RPE cells when challenged by oxidative stress
TIMP (TIMP-1 and TIMP-3)	Involved in extracellular matrix degradation	Plays a role in preneovascularization
Pigment epithelium-derived factor	Antiangiogenic, anti-tumorigenic, and neurotrophic protein	Fails to inhibit cell proliferation and maintain the retinal structure
Ciliary neurotrophic factor	Interferes with MAPK, PI3K, and NF-κB signaling pathways	Increases RPE cell survival
Fibroblast growing factor	Induces VEGF expression in retinal vascular cells	May represent a source of neural regeneration

Note: Data adapted from Weight et al.[58]
Abbreviations: DR, diabetic retinopathy; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PI3K, phosphoinositide 3-kinase; RPE, retinal pigment epithelium; TIMP, tissue inhibitor of matrix metalloproteinase; VEGF, vascular endothelial growth factors.

maintain the ocular vasculature under control. These proteins modulate cellular proliferation, migration, differentiation, and apoptosis and influence the oxidative state and phagocytic activity of RPE cells.

TSP-1 induced angiogenesis in vivo at low concentrations,[64,65] whereas in higher concentrations, inhibition occurred. This homotrimeric matricellular glycoprotein is produced by various cell types. At retinal level, it supports RPE cell structure and inhibits vascular endothelial cell adhesion.[66] TSP-1 production in the eye is dramatically reduced in diabetes.[75]

PEDF is found in the human eye from early embryonic stages,[58] and different studies confirm its presence in RPE cells.[67–69] This homotrimeric matricellular glycoprotein proved to be a more potent inhibitor of endothelial cell migration caused by angiogenic inducers compared with TSP-1 and angiostatin;[70] one of its mechanisms includes the inhibition of NADPH oxidase activity induced by AGEs.[71,72]

Sheibani et al demonstrated that high glucose levels affect TSP-1 production by endothelial cells in vitro with the appearance of retinal vasculopathies.[73] An in vivo study performed on Akita/+ male mice deficient in TSP-1 demonstrated an increase in pathological vascular changes; in addition, Akita/+ PEDF-deficient mice also showed an increase in acellular capillaries.[74] Other previous studies measured the levels of TSP-1 and PEDF from vitreous samples of diabetic and nondiabetic patients. The diabetic group had lower levels of TSP-1 and a higher molecular weight PEDF isoform.[75]

PEDF has been showed to inhibit the development of DR, proving to be a good candidate for the treatment of DR. However, practical pharmaceutical applications are limited due to its structure. Finding a natural compound which could modulate its expression might be a solution to rebalance the oxidant status of the cell.

Identification of new mechanisms of action for natural antiangiogenic compounds is very important and the normal step to take in case of resistance to long-term use of angiogenic inhibitors.

Natural therapeutic agents

It is important, when considering a new therapeutic strategy, to target a mechanism independent of VEGF since present anti-VEGF drugs have shown similar efficacy and only an independent mechanism of action could improve outcomes, rather than an additional suppression of this pathway.

Resveratrol, or 3,5,4′-trihydroxystilbene, is a phytoalexin produced by plants as a response to stress, fungal infections, injuries, or ultraviolet radiation. This polyphenol belongs to the class of stilbenes and has been isolated for the first time from *Veratrum grandiflorum*. It has made the subject of >50,000 scientific articles over the past decade only, and its ability to target intracellular molecules and processes is being intensively studied. *Polygonum cuspidatum*, *Vitis vinifera*, different *Vaccinium* species, and *Arachis hypogaea* are among the known sources of resveratrol.[76]

Resveratrol modulates various pathways, including PKC,[77] rebalances the cellular oxidative status,[78,79] and is proved to be a powerful activator of sirtuin 1 (SIRT-1), an angiogenesis key regulator.[80] In vitro and in vivo studies showed multiple mechanisms of action of resveratrol, with a preponderant targeting of angiogenic factors: it reduces VEGF accumulation via the activation of AMPK,[81] suppresses TGF-β2-induced cell migration in ARPE-19 cells,[82] and inhibits PDGF-induced RPE cell migration,[83] slowing the process of degradation of the blood–retina barrier. A double-blind, randomized, placebo-controlled study on 44 healthy subjects revealed that a daily intake of 400 mg of resveratrol for a month reduced endothelial activation and vascular inflammation quantified by the reduction in the expression of interleukin-8 and cell adhesion molecules (ICAM-1 and vascular cell adhesion molecule-1).[84] In addition, daily administration of 8 mg of resveratrol for 6 months to patients undergoing primary cardiovascular disease prevention, followed by an increase to 16 mg of resveratrol for another 6 months, significantly reduced the levels of C-reactive protein, tumor necrosis factor-α, and PAI-1, improving the inflammatory status.[85]

Published literature documents the inhibitory effect of resveratrol on pro-angiogenic protein molecules. Research on the influence of resveratrol on antiangiogenic cytokines is scarce. One study was performed on human retinal pigment epithelial cells and failed to reveal a positive influence on PEDF secretion (enzyme-linked immunosorbent assay).[86] Another study explored SIRT-1 activation in Müller glial cells and found that resveratrol enhances antiangiogenic factors by increasing gene expression of PEDF and TSP-1 by 1.4-fold and 1.5-fold, respectively. Respiratory syncytial virus upregulation of PEDF and TSP-1 may have antichoroidal neovascularization properties.[87] A high-glucose environment decreases SIRT-1 activity and promotes matrix metalloproteinase-9 (MMP-9) activation through NF-κB pathway.[88] MMPs contribute to the development of tissue injury and inflammation at various sites, including the retina.[89] A study using a mouse model of retinopathy investigated MMP effects on PEDF proteolysis and found that it abolishes PEDF protective activity and retinal survival.[90] Knowing the neuroprotective role of resveratrol against

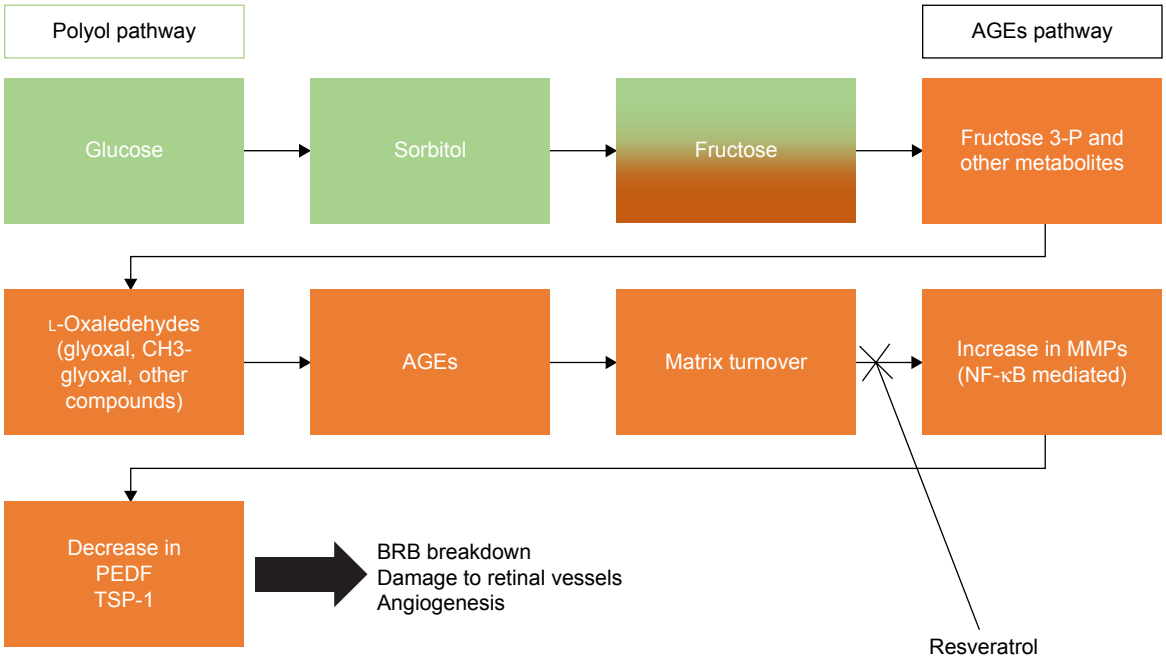

Figure 7 Schematic presentation of a possible mechanism of action for resveratrol in the development of diabetic retinopathy.
Abbreviations: AGEs, advanced glycation end-products; BRB, blood–retinal barrier; MMPs, matrix metalloproteinases; NF-κB, nuclear factor-κB; PEDF, pigment epithelium-derived factor; TSP-1, thrombospondin-1.

ischemia, its interaction with MMPs was studied, and it revealed an inhibitory activity by interaction with MMP active site residues.[91] A possible pathway to investigate could be the enhancement of PEDF and TSP-1 by resveratrol through the inhibition of MMPs, bearing in mind that PEDF expression increases TSP-1 production[92] and promotes retinal survival. Figure 7 illustrates possible mechanisms of action for resveratrol in the development of DR.

Delivery systems overview on oral pharmaceutical forms

Resveratrol is a chemically instable compound with poor water solubility, being well absorbed (~75%), but with a low bioavailability (<1%),[93] which limits its activity and biological effects. Following oral administration, the biological half-life is short (8–14 minutes), and in vitro evidence revealed undetected levels in targeted tissues.[94]

Short-term studies reported minor adverse effects, most of them being gastrointestinal side effects.[95] Still, the large number of in vitro effects of free resveratrol remains controversial, since it is rapidly metabolized, achieving a negligible concentration in the bloodstream.

Lately, numerous formulations have been investigated to increase the solubility and the bioavailability of resveratrol or to obtain targeted/prolonged delivery.[96–98] Those formulations contain different forms of resveratrol varying from pro-drug administration to nanodelivery systems.[94] Among the large variety of nanoformulations reported, liposomal preparations are biocompatible because the addition of organic solvents such as DMSO in the preparation step is excluded.[100] Encapsulation of the active substance in liposomes prevents the inactivation through *cis–trans* isomerization and leads to an improved chemical stability of resveratrol.[100,102] Several studies showed a targeted action for liposomes, resulting in an enhanced delivery with superior efficacy compared with free compounds.[100,103,104]

Moreover, combined delivery systems such as liposomes loaded with resveratrol encapsulated in alginate, alginate–sucrose, and alginate–chitosan microbeads[98] and liposomes loaded with resveratrol–cyclodextrin inclusion complexes[100] were reported, as having an improved drug release profile. Nanoemulsions have been developed to enhance the solubility and bioavailability of lipophilic compounds or to protect the antioxidant properties of resveratrol.[101,112,113] According to Sessa et al, nanoemulsions sustain the controlled release of the encapsulated resveratrol,[101] along with the increase of its half-life.[114] Recently, self-emulsifying drug delivery systems have been documented to increase the solubility and bioavailability of resveratrol[96] and to enhance the intestinal permeation.[115]

Other carriers, such as nanoparticles, were extensively studied, and different systems for the delivery of resveratrol

have been designed. Solid nanoparticles were prepared using protein molecules such as zein,[99] gliadin,[105] β-lactoglobulin,[106] gelatin,[110] or other biodegradable polymers such as poly(DL-lactide-co-glycolide)[107] and polyethylene glycol–polylactic acid.[108,109] Encapsulation of the drug in nanoparticles protects it against degradation, increases bioavailability, and ensures sustained release. Solid lipid nanoparticles and nanostructured lipid carriers have been proposed as alternative carriers for targeted delivery of resveratrol, showing advantages such as stability and high entrapment efficiency over classical nanoemulsions and liposomes.[97,111]

To date, a large number of nanodelivery systems have been studied, but more research is needed to transfer in vivo all potential beneficial effects of resveratrol. An ideal delivery system for resveratrol should increase solubility, stability, and bioavailability, should prolong release with targeted delivery to improve its therapeutic efficiency, and should reduce the doses administered.

Summary and future directions

There is an increasing interest toward natural compounds, especially those presenting antioxidant, anti-inflammatory, or immunomodulatory properties. Resveratrol is of high interest for its many mechanisms of action that can provide researchers with new data in molecular biology. DR is a priority eye disease on the VISION 2020 list, for prevention and treatment. Despite laser photocoagulation as the standard care, intravitreal drugs as alternative options, and vitreoretinal surgery for severe cases of DR, this vision-threatening condition remains a continuous clinical challenge.

This review aimed at underlining the complexity of the pathogenesis of diabetic microvascular complications, with four main mechanisms being directly responsible for the oxidant/antioxidant cellular imbalance and tissue damage. Each pathway contains intermediate products produced through other reactions involved in this pathological process. The retinal secretome is sensitive to glycemic fluctuations, and because of the accumulation of deleterious and highly reactive products, proteins secreted in the retina are overproduced or inhibited depending on their overall actions.

The need for targeting new mechanisms in the development of DR is more evident than ever. The retina produces pro-angiogenic factors as well as protective molecules in its attempt to balance the cellular media and to assure the tissue survival. This review underlines the importance of further studying natural protective agents and how they can influence or even rise the levels of "good" proteins in specific tissues that can protect our eyes from diabetic complications.

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Disclosure

The authors report no conflicts of interest in this work.

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