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REVIEW ARTICLE

Neurodegeneration in diabetic retinopathy: Current concepts and therapeutic implications

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Abstract Diabetic retinopathy (DR), the most common complication of diabetes and one of the leading causes of preventable blindness, has been considered to be a microcirculatory disease of the retina. However, there is emerging evidence to suggest that retinal neurodegeneration is an early event in the pathogenesis of DR, which participates in the development of microvascular abnormalities. Therefore, the study of the underlying mechanisms leading to neurodegeneration and the identification of the mediators linking neurodegeneration and microangiopathy will be essential for the development of new therapeutic strategies in the early stages of DR. In this review the mechanisms involved in neurodegeneration, as well as the link between neurodegeneration and microangiopathy have been updated. Finally, the therapeutic implications and new perspectives based on identifying those patients with retinal neurodegeneration are presented.

PALABRAS CLAVE
Retinopatía diabética;
Neurodegeneración;
Neuroprotección;
Microangiopatía diabética;
Unidad neurovascular;
Colirio

Neurodegeneración en la retinopatía diabética: estado actual del tema e implicaciones terapéuticas

Resumen La retinopatía diabética (RD), la complicación más frecuente de la diabetes y una de las principales causas de ceguera, se ha considerado clásicamente como una enfermedad microangiopática de la retina. Sin embargo, la evidencia actual indica que la neurodegeneración es un fenómeno precoz en la patogenia de la RD que participa en el desarrollo de las alteraciones microvasculares. Por tanto, el estudio de los mecanismos que ocasionan la neurodegeneración y la identificación de los mediadores involucrados en la asociación neurodegeneración y microangiopatía serán esenciales para plantear nuevas estrategias terapéuticas en las fases iniciales de la RD. En esta revisión se exponen los conocimientos actuales sobre la relación entre alteraciones neurodegenerativas y microvasculares de la retina. Finalmente, se comentan las

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implicaciones terapéuticas así como las nuevas perspectivas en el manejo clínico de esta complicación a partir de la identificación de los pacientes diabéticos con neurodegeneración de la retina.

Diabetic retinopathy (DR) is the leading cause of visual impairment and preventable blindness,[1,2] and represents a significant socio-economic cost for healthcare systems worldwide.[1] DR prevalence in the diabetic population is around one-third and one-tenth has vision-threatening states such as diabetic macular oedema (DME) or proliferative diabetic retinopathy (PDR).[2] DR has been classically considered to be a microcirculatory disease of the retina. However, there is growing evidence to suggest that retinal neurodegeneration is an early event in the pathogenesis of DR which participates in the microcirculatory abnormalities that occur in DR.[3–5]

Current treatment of diabetic retinopathy

Tight blood glucose levels and blood pressure control are essential in preventing DR development or arresting its progression. When DR appears, the present standard of care relies on laser photocoagulation, which is inherently destructive, associated with unavoidable side effects (i.e. visual field loss and impairment of either dark adaptation or colour vision), and not universally effective in reversing or preventing visual loss.[6] Intravitreal corticosteroids have been successfully used in eyes with persistent DME and loss of vision following the failure of conventional treatment. However, reinjections are commonly needed, and there are substantial adverse effects such as infection, glaucoma and cataract formation.[7] In recent years intravitreal anti-VEGF agents have emerged as new treatments for more advanced stages of DR. Several trials have provided robust evidence that intraocular administration of anti-VEGF agents is superior to laser therapy in preserving and improving vision for patients with DME.[8–10] However, this is an invasive procedure, which may lead to complications such as endophthalmitis, retinal detachment and could even have deleterious effects for the remaining healthy retina. This is especially important in diabetic patients in whom long-term administration is to be expected. Apart from local side effects, anti VEGF agents could also produce systemic complications due to their capacity to pass into systemic circulation. Therefore, specific studies in diabetic patients on the long term effectiveness and safety of intravitreal anti-VEGF agents are still needed.[11] Vitreo-retinal surgery is an expensive treatment that should be carried out only by vitreoretinal specialists experienced in this procedure and it is normally reserved for the ultimate blinding complications of PDR.[12]

In summary, current treatments for DR are applicable only at advanced stages of the disease and are associated with significant adverse effects. Therefore, new pharmacological treatments for the early stages of the disease are needed.

Neurodegeneration in the diabetic eye

Histological findings and signalling pathways leading to apoptosis

Although microcirculatory impairment is the classic hallmark of DR, there is emerging evidence to suggest that retinal neurodegeneration is an early event in the pathogenesis of DR which participates in the microcirculatory abnormalities that occur in DR.[3–5] In this regard, it is worth mentioning that the main features of retinal neurodegeneration (apoptosis and glial activation) have been found in the retinas of diabetic donors without any microcirculatory abnormalities appearing in the ophthalmoscopic examinations performed during the year before death.[13–15] Therefore, a normal ophthalmoscopic examination does not exclude the possibility that retinal neurodegeneration is already present in the diabetic eye (Fig. 1).

Retinal ganglion cells (RGCs), located in the inner retina, are the retinal neurons in which the apoptotic process related to diabetes is first detected.[16] This loss of neural cells results in a reduction in the thickness of the retinal nerve fibre layer.[17,18] It should be noted that this thinning of the RGC layer has been found in diabetic patients without or with only minimal DR.[16–19] In addition, it has been recently demonstrated that in the early stages of DR an imbalance between proapoptotic and survival signalling exists in the neuroretinas of diabetic patients.[20]

Neural apoptosis is accompanied by reactive changes in both types of glial cells (microglia and macroglia), the most representative being those occurring in macrogial cells. The retina has two types of macroglial cell. The predominant type is the Müller cell, which is unique to the retina. Müller cells are spindle-shaped and span the entire retina from the outer limiting membrane to the retinal ganglion cells. The second type is the astrocyte, which migrates into the retina along the optic nerve during development. Astrocytes are less abundant than Müller cells and form a monolayer at the inner limiting membrane. Retinal astrocyes normally express GFAP (Glial fibrillar acidic protein), while in Müller cells this expression is much lower. However, in diabetes an aberrant expression of GFAP is shown by Müller cells.[21] Because Müller cells produce factors capable of modulating blood flow, vascular permeability, and cell survival, and their processes surround all the blood vessels in the retina it seems that these cells play a key role in the pathogenesis of retinal microangiopathy in the diabetic eye.[22]

Electroretinogram abnormalities

The electroretinogram (ERG) is one of the most important tools currently used for exploring functional abnormalities

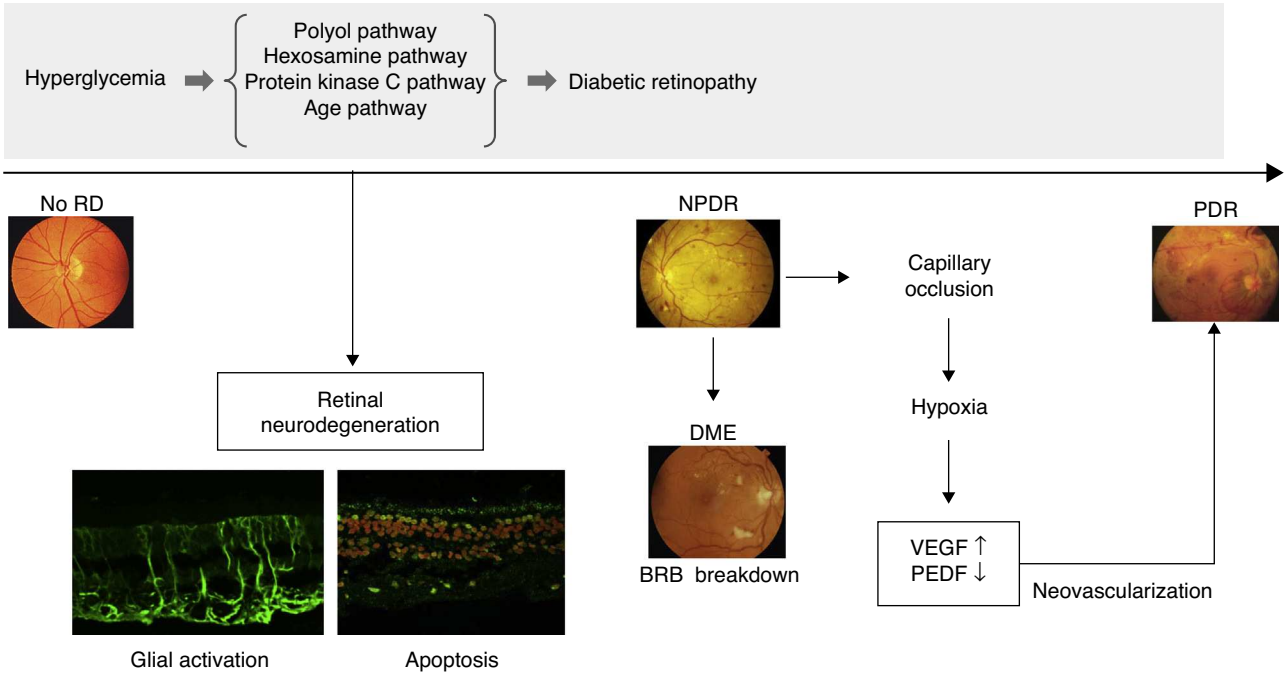

Figure 1 Schematic representation of DR development. The metabolic pathways triggered by hyperglycemia, and hyperglycemia itself, lead to DR but a period of at least 5 years is required before DR can be diagnosed under ophthalmoscopic examination. The first stage that we can see is background retinopathy or non-proliferative diabetic retinopathy (which is constituted by microaneurysms, microhemorrhages and hard exudates). From this stage the natural history of the disease can follow two directions that do not exclude the other. One of them is the development of clinically significant macular oedema in which the most important pathogenic element is the breakdown of the blood-retinal barrier (BRB). This way is more frequent in type 2 diabetic patients. The other direction is towards PDR which is more frequent in type 1 diabetes. In this setting capillary occlusion plays an essential role generating an imbalance between angiogenic and antiangiogenic factors, which finally stimulates neovascularisation (the hallmark of PDR). However, even before NPDR could be detected in the ophthalmologic examination, retinal neurodegeneration does exist. At the bottom it is a representative case of type 2 diabetic donor without any abnormality in the funduscopy performed in the preceding year before death. Left panel: immunostaining with GFAP showing glial activation. Right panel: TUNEL immunostaining showing a high rate of apoptosis.

secondary to the neurodegenerative process that occurs in the diabetic eye. The ERG has several characteristic components which are altered in both human and diabetic murine models. A reduction in the amplitude and a delay in the latency of the oscillatory potentials have been found in both diabetic patients and rats without any evidence of microvascular abnormality.[23–26]

The use of multifocal ERG (mfERG) has provided compelling evidence suggesting a direct link between neural dysfunction and vascular abnormalities in DR. The mfERG is a technique for assessing the local ERG from different regions of the retina. Electrical responses from the eye are recorded with a corneal electrode just as in conventional ERG recording, but the special nature of the stimulus and analysis produces a topographic map of ERG responses. A delayed mfERG implicit time (mfERG-IT) predicts the development of early microvascular abnormalities.[27–31] The implicit time in mfERG (elapsed time from the stimulus to P1 peak) is spatially associated with DR, correlates with DR severity and is a predictor for the development of visible vascular abnormalities over a 1-year,[29,30] and a 3-year period.[31] In addition, this spatial coincidence might also suggest that neuronal death or dysfunction leads to vascular damage. In this regard, it has been recently reported that diabetic patients without structural microvascular abnormalities have a reduced vasodilation response to flicker light stimulation.[32,33] Furthermore, it has been suggested that flicker light-induced vasodilation is mediated primarily by ganglion cells whose function is strongly correlated with the ERG pattern.[34] All these findings reinforce the concept that neurodegeneration plays an essential role in early microvascular abnomalities that occur in the diabetic eye.

Mediators of retinal neurodegeneration

Extracellular glutamate accumulation, oxidative stress and reduction of neuroprotective factors synthesized by the retina are all involved in the neurodegenerative process that occurs in DR and plays an essential role in its pathogenesis.

Extracellular glutamate accumulation

Glutamate is the major excitatory neurotrasmitter in the retina and it has been found elevated in the extracellular space in experimental models of diabetes,[35–37] as well as

in the vitreous fluid of diabetic patients with PDR.[38,39] This extracellular and synaptic excess of glutamate leads to overactivation of ionotropic glutamate receptors, mainly alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and N-metil-D-aspartame (NMDA) receptors, which results in an uncontrolled intracellular calcium response in postsynaptic neurons and cell death.[40,41] This deleterious effect of glutamate on retinal neurons is known as ''excitotoxicity''.

The reasons why diabetes facilitates extracellular accumulation of glutamate includes: (1) Increase of glutamate production by glial cells due to the loss of the Müller cell-specific enzyme glutamine synthetase, which converts glutamate to glutamine.[35,36] (2) Reduction in the retinal ability to oxidize glutamate to alpha-ketoglutarate.[36] (3) Impairment of glutamate uptake by the glial cells. An essential step in the regulation of extracellular glutamate is the transport of this amino acid into Müller cells through the high-affinity L-glutamate/L-aspartate transporter (GLAST) which has been compromised in the diabetic retina.[42]

Oxidative stress

The molecular mechanisms of hyperglycemia-induced DR are not fully clear, and the majority of publications focus on multiple biochemical pathways, including the augmentation of polyol pathway, protein kinase C (PKC) activation, increased advanced glycation endproducts (AGEs) formation, the receptor for AGEs and its activating ligands, and overactivity of the hexosamine pathway. However, all these mechanisms are activated by a single event: the aberrant production of the mitochondria-derived reactive oxygen species (ROS) to increase the level of oxidative stress.[43]

The retina is the only neural tissue that has a direct and frequent exposure to light. This result in the photooxidation of many lipids, especially polyunsaturated fatty acids and cholesterol esters, and these oxidized lipids become extremely toxic to retinal cells.[44] In DR, this problem is aggravated by the increase of oxidative stress and lipid peroxidation associated with diabetes.

There is emerging evidence that oxidative stress is able to damage both neural (in particular RGCs) and microvascular retinal cells.[45,46] One of the mechanism by which oxidative stress leads to neuronal death is through the impairment of GLAST (the main transporter for removing glutamate from extracellular space).[42] Therefore, antioxidant therapy is being studied to prevent induction of the various pathogenic mechanisms of DR.

Imbalance in the retinal production of neuroprotective factors

The retinal production of several neuroprotective factors such as pigment epithelial derived factor (PEDF), somatostatin (SST) and interstitial retinol-binding protein (IRBP) has been found lower in the retina of diabetic patients than in non-diabetic subjects. The downregulation of these factors can reduce the neuroprotection against neurotoxic factors (i.e. glutamate and oxidative stress) involved in neurodegeneration. Therefore, several strategies addressed to replacing these natural neurorptotective factors are currently under investigation.

PEDF is a potent neuroprotective and anti-angiogenic factor that is downregulated in DR.[47] It protects retinal neurons from light damage, oxidative stress and glutamate excitotoxicity.[48]

Somatostatin (SST) is also an endogenous peptide synthesized by the retina with antiangiogenic and neuroprotective properties.[47] In the human retina the main source of SST is RPE,[13] and the amount of SST produced is significant as can be deduced by the strikingly high levels found in the vitreous fluid.[49–51] In both PDR and DME there is a lower production of SST which results in a significant decrease of its intravitreal levels.[49–51] In addition, it has been reported that the downregulation of SST production by the human retina occurs at very early stages of DR and it is associated with retinal neurodegeneration.[13]

A low expression and content of interstitial retinol-binding protein (IRBP) has been reported in the retinas from diabetic donors at very early stages of DR, and this downregulation was associated with retinal neurodegeneration.[15] IRBP is a large glycoprotein synthesized by the photoreceptors and extruded into the interphotoreceptor matrix that fills the subretinal space.[52,53] Apart from participating in the visual cycle, IRBP is important in fatty acid transport and is essential to the maintenance of the photoreceptors.[54,55] In this regard, a reduction of IRBP may precede the loss of photoreceptors seen in some animal models of hereditary retinal degeneration.[56,57] In addition, knockout ($Irbp^{-/-}$) mice revealed a loss of photoreceptors and profound changes in the structural integrity of the receptor outer segments.[55,58] Finally, a homozygous missense mutation in the *IRBP* gene has been associated with autosomal recesive retinitis pigmentosa in children.[59] For all these reasons IRBP replacement can be contemplated as a new therapeutic strategy for DR.

Apart from the downregulation of natural neuroprotective factors produced by the retina an upregulation of neurothropic and survival factors such as vascular endothelial growth factor (VEGF) and erythropoietin (Epo) also exist in the diabetic retina. Notably, this overexpression is already detected in early stages of DR and is not related to hypoxia.[60,61] Therefore, stimulating agents other than hypoxia/ischaemia are involved in the upregulation of VEGF and Epo that exists in the diabetic eye.

VEGF is a well-known pathogenic factor for DME and PDR but it has also significant neurotrophic and neuroprotective properties.[62,63] The secretion of VEGF by RPE is essential for choriocapillaris development (the vascular network that underlies the retina) and has a neuroprotective effect in the ischaemic retina.[60] In this regard, a dose-dependent decrease in RGCs has been reported following the injection of an antibody that blocks all VEGF isoforms in rats.[64] Furthermore, this loss of neural cells is apparent prior to any observable effect on the vasculature. However, other experimental studies have not found significant neural damage in VEGF knockout mice[65] or after blocking phosphorylation of VEGF receptors in transgenic mice with sustained expression of VEGF in photoreceptors.[66] These findings could have clinical implications since, so far, clinical trials using anti-VEGF treatment have focused only on studying the systemic side effects, such as cardiovascular, hypertension, proteinuria, or bleeding but not the incidence of retinal neurodegeneration, such as retinal atrophy or RPE degeneration.[11]

Therefore, further reseach on this issue is urgently needed.

Epo is also a potent neuroprotective factor,[67–69] and strikingly high levels have been found in the vitreous fluid of diabetic patients (~30 fold-higher than plasma and ~10 fold higher than in non-diabetic subjects).[70] In recent years, it has been demonstrated that not only Epo but also its receptor (Epo-R) is expressed in the adult human retina.[61] These findings point to Epo as a natural neuroprotective factor with autocrine/paracrine actions in the retina. Apart from neuroprotection, Epo is a potent physiological stimulus for the mobilization of endothelial progenitor cells (EPCs) towards injured retinal sites, thus participating in the remodelling of the damaged tissue.[71] It should be underlined that in the human retina hypoxia is not a crucial element for the upregulation of Epo because intravitreal levels of Epo have been found at a similar range in both PDR and DME (a condition in which hypoxia is not a predominant event).[70] In addition, intravitreal Epo levels are not elevated in non-diabetic patients with macular oedema secondary to retinal vein occlusion.[72] Finally, the overexpression of Epo detected in the retinas from diabetic donors at early stages of DR in comparison with non-diabetic donors is unrelated to mRNA expression of hypoxic inducible factors.[61]

The overexpression of these factors counteracts the reduction of neuroprotective factors above mentioned and, therefore, plays a key role in restoring the neural damage induced by the diabetic milieu in the early stages of DR. However, in advanced stages of DR the elevated levels of either VEGF or Epo could favour neovascularization, thus contributing to PDR development.[71,73] In addition, Epo could enhance the effects of VEGF. Therefore the overxpression of VEGF and Epo might act as a double-edged sword in the pathogenesis of DR.

Other neuroprotective factors such as insulin, neuroprotectin D1 (NPD1), brain-derived neurotrophic factor (BDNF), glial cell-line-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF), nerve growth factor (NGF) and adrenomedullin (AM) might also be involved in the neurodegenerative process that occurs in DR but specific studies on this issue are still needed.

Other contributing factors

A large body of evidence supports the role of proinflammatory cytokines, chemokines, and other inflammatory mediators in the pathogenesis of DR leading to persistent low-grade inflammation which contributes to the damage of retinal vasculature.[74,75] An emerging issue in DR research is the focus on the mechanistic link between activation of sublinical inflammation and neurodegeneration. In this regard, it has been shown that Müller cells show inflammation-linked responses when exposed to the diabetic milieu.[76,77] In addition, it has recently been demonstrated that upregulation of the receptor for AGEs (RAGE) plays a key role in hyperglycaemia-induced activation of Müller glia and downstream cytokine production in the context of DR.[78,79] The mechanism by which these cytokines may contribute neural apoptosis is not clear but may involve the induction of excitotoxicity, oxidative stress, or mitochondrial dysfunction.[80]

Finally, there is emerging evidence that renin-angiotensin system (RAS) activation[81–84] plays an essential role in the retinal neurodegeneration induced by diabetes.

Mechanisms linking retinal neurodegeneration with microvascular abnormalities

Emerging evidence suggests that neurodegeneration participates in early microvascular changes that occur in DR such as the breakdown of the blood–retinal barrier (BRB), vasoregression and the impairment of neurovascular coupling.[5]

Neurovascular coupling is the intrinsic physiological mechanisms by which neural activity is coupled to blood flow and metabolism, thus enabling the retina to regulate blood flow in response to neural activity or metabolic demands. Visual stimulation is a powerful modulator of retinal and optic nerve blood flow[34] and flicker light stimulation (intermitent flash) has been used to investigate this process because it increases neural activity. This increase of neural activity leads to retinal arterial and venous dilation[85] because of the release of vasodilating factors, especially nitric oxide (NO), from neural cells and endothelial cells.[86] Flicker-induced retinal diameter change has been shown to deteriorate early in patients with diabetes.[32,87]

Therapeutic implications

Treatment based on neuroprotection opens up a new approach for preventing or arresting DR development. From the clinical point of view, the identification of those patients in whom retinal neurodegeneration appears will be crucial for implementing an early treatment based on drugs with a neuropotective effect. This treatment would not only arrest the progression of retinal neurodegeneration but also prevent the development and progression of the early stages of DR (i.e. microaneurysms and/or retinal thickness).

The reduction of oxidative stress and the administration of neuroprotective agents are among the most important therapeutic strategies based on neuroprotection. There are several pharmacological studies showing that reducing oxidative stress may be an effective approach to slow neurodegeneration in experimental DR.[84,88] Neuroprotective factors such as PEDF, SST and Epo have been used in experimental research. Intraocular gene transfer of PEDF significantly increases neuroretinal cell survival after ischaemia-reperfusion injury and excessive light exposure. In addition, PEDF protects neurons from glutamate-mediated neurodegeneration. SST and SST analogues administered intravitreally protect the retina from AMPA-induced neurotoxicity.[89] Exogenous Epo administration by intravitreal[90] or intraperitoneal injection[91] in early diabetes may prevent structural vascular and neural damage in STZ-DM rats. Nevertheless, in advanced stages the elevated levels of Epo could enhance the effects of VEGF, thus contributing to neovascularisation and, in consequence, worsening PDR.[71,73]

As different phases in ocular diabetes-related abnormalities have been extensively reported, research should now evaluate at what stage in the course of DR new pharmacological approaches may be optimally instituted. However, in the early stages of DR it is inconceivable

that an aggressive treatment such as intravitreal injections would be recommended, and there is emerging evidence that many drugs are able to reach the retina in pharmacological concentrations, at least in animal models.[92] In fact, the neuroprotective effects of topical administration of brimonidine, NGF and SST have already been reported in experimental models.[93–95] In addition, the topical administration of drugs limits their action to the eye and minimizes the associated systemic effects, resulting in higher patient compliance.[96] Therefore, topical therapies could revolutionize the care of diabetic patients.[1] Notably, a multicentre, phase II-III, randomized controlled clinical trial (EUROCONDOR-278040) to assess the efficacy of two neuroprotective agents (SST and brimonidine) administered topically to prevent or arrest DR was approved by the European Commission in the setting of the FP7-HEALTH-2011 and is already ongoing.

Concluding remarks and future perspectives

Neurodegeneration plays a key role in the pathogenesis of DR and, therefore, therapeutic strategies based on neuroprotection could open up a new strategy for treatment of the early stages of DR.

The functional abnormalities indicative of neuroretinal damage detected by mfERG could be correlated with structural changes occurring in the retina using Frequency Domain Optical Coherence Tomography (FD-OCT). It will be of particular interest to evaluate changes in the nerve fibre layer, ganglion cell density, photoreceptor abnormalities, retinal thickness, and the quantification of the extracellular space of the retina. In fact, based on these two examinations, it would be possible to identify diabetic patients with neurodegeneration and, therefore, those patients in whom neuroprotection should be implemented. Although mfERG and FD-OCT allow us to monitor neurodegeneration, standardization processes will be required their widespread use in clinical practice can be encouraged. In addition, it could be argued that this new proposed screening method for detecting DR would be too expensive for health-care systems.

In conclusion, the central role of neurodegeneration in the pathogenesis of DR is a solid basis for proposing neuroprotection as an effective strategy for preventing or arresting DR. However, clinical trials to determine not only the effectiveness and safety but also the compliance of a non-invasive route to administer these drugs, as well as a standardization of the methods for monitoring neurodegeneration such as mfERG and FD-OCT are needed.

Conflict of interest

The authors declare no conflict of interest.

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