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REVIEW

Diabetic retinopathy: hyperglycaemia, oxidative stress and beyond

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Abstract Diabetic retinopathy remains a relevant clinical problem. In parallel with diagnostic and therapeutic improvements, the role of glycaemia and reactive metabolites causing cell stress and biochemical abnormalities as treatment targets needs continuous re-evaluation. Furthermore, the basic mechanisms of physiological angiogenesis, remodelling and pruning give important clues about the origins of vasoregression during the very early stages of diabetic retinopathy and can be modelled in animals. This review summarises evidence supporting a role for the neurovascular unit—composed of neuronal, glial and vascular cells—as a responder to the biochemical changes imposed by reactive metabolites and high glucose. Normoglycaemic animal models developing retinal degeneration, provide valuable information about common pathways downstream of progressive neuronal damage that induce vasoregression, as in diabetic models. These models can serve to assess novel treatments addressing the entire neurovascular unit for the benefit of early diabetic retinopathy.

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Abbreviations
BRB	Blood–retinal barrier
EDIC	Epidemiology of Diabetes Interventions and Complications
GLP-1	Glucagon-like peptide 1
iNOS	Inducible NO synthase
ROS	Reactive oxygen species
STZ	Streptozotocin
UKPDS	UK Prospective Diabetes Study
VEGF	Vascular endothelial growth factor

Diabetic retinopathy: the danger is not over

The overall prevalence of diabetic retinopathy is 35% among people with diabetes worldwide, with a current decline in both any retinopathy and sight-threatening stages [1]. Diabetic retinopathy ranks fifth among common causes for blindness or severe vision impairment and a recent meta-analysis revealed that the age-standardised prevalence of diabetic retinopathy-related blindness will increase due to increasing populations and average age together with a reduction in death rates [2]. Lending support to the magnitude of the problem are reports that even when a person first presents at screening services, advanced diabetic retinopathy is present at levels that are no longer amenable to medical interventions [3, 4]. Recent population-based studies from Europe revealed that screening-detected diabetic retinopathy appeared in more than

Background

- Diabetic retinopathy is a prevalent clinical problem

- Diabetic retinopathy affects the neurovascular unit, not only the vasculature

- Physiological processes such as angiogenesis and remodelling are instructive for understanding retinopathy, as are euglycaemic animal models with retinal degeneration

- Animal models of diabetes reflect preclinical human disease but not diabetic macular oedema or proliferative diabetic retinopathy

13% of individuals with newly diagnosed type 2 diabetes [4]. Although lower than the rate reported from the UK Prospective Diabetes Study (UKPDS) [5], diabetes is still underdiagnosed and individuals may present when retinopathy is beyond the 'point of no return' at which progression can be halted by glucose-lowering therapy (see Fig. 1).

Diabetic retinopathy is classified as a microvascular disease and has been empirically lumped together with diabetic nephropathy and neuropathy for decades. However, modern technologies, such as multifocal electroretinography and microperimetry, which assess neuroglial function in correlation with the structure of the human diabetic retina, have allowed diabetic retinopathy, with its complex pathology and divergent dynamics and disparities, to be distinguished from nephropathy and neuropathy [6–9]. Discussion of the risk factors relevant to
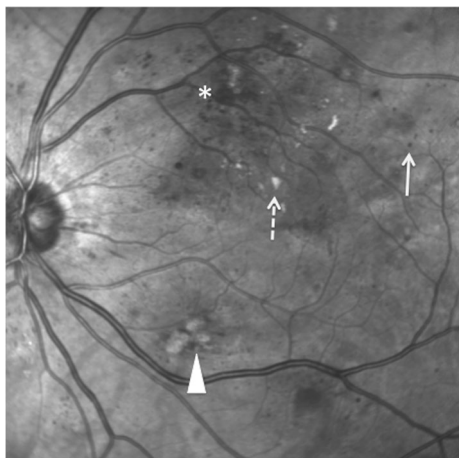
Fig. 1 Diabetic retinopathy in a male patient with newly discovered type 2 diabetes (known diabetes duration 6 months; no signs of neuropathy and nephropathy). The fundus (left eye) shows microaneurysms (solid arrow), haemorrhages (asterisk) and hard (dashed arrow) and soft (arrowhead) exudates. Hard exudates are found within one disk diameter around the fovea, classifying this stage as severe non-proliferative (all four quadrants of the retina affected) diabetic retinopathy with central macular oedema

specific damage to target tissues may help to explain these disparities. Moreover, chronic hyperglycaemia, multiple risk factors, including sex, genetics, disease duration, blood pressure and lipids, have been identified and reviewed extensively [10, 11]. Of note, obstructive sleep apnoea as a frequent comorbidity in type 2 diabetes has been recently recorded as an aggravating factor and may benefit from specific airways treatment [12].

Retinal vasculature: from development to early changes in diabetes

To understand the evolution of incipient diabetic retinopathy, the development of the retinal vasculature and its relation to the microenvironment must be decoded and appreciated. Two vascular plexuses develop in the eyes of humans and some animals: the choroid (supplying 90% of the total blood volume to the retina) and the intraretinal vasculature (supplying the inner-third of the retina, sparing the macula), which is divided into a superficial and a deep plexus [13, 14].

The elaborate retinal network is the result of a highly coordinated process during development. In humans, the retinal vasculature forms during late embryogenesis, while in mice it forms postnatally, facilitating the study of basic mechanisms of angiogenesis [13, 15, 16]. The superficial vascular layer of the retina is formed on a template of astrocyte glia, which produce a gradient of vascular endothelial growth factor (VEGF) spreading radially towards the edge of the retina. Around day 7, the vasculature starts a perpendicular growth towards the inner nuclear layer (potentially following a VEGF gradient produced by the developing rod outer limbs), forming a second and third vascular plexus. Subspecialisation of endothelial cells is essential to the angiogenic process: endothelial tip cells direct sprouting vessels along a gradient of VEGF; endothelial stalk cells proliferate and start attracting pericytes and endothelial phalanx cells represent a quiescent phenotype of endothelial cells that do not proliferate and are less sensitive to regressive signals [17]. This elaborate effort results in an almost identical retinal capillary phenotype in animal models (mouse, hamster, rat, monkey) when compared with the human retina (Fig. 2a–f). Using quantitative retinal morphometry, the number of endothelial cells and pericytes in retinal capillaries can be assessed; the highest density of endothelial cells occurs in mice and the lowest in humans (Fig. 2g).

The macula is only present in the retinas of humans and some primates. Hence, there is no rodent model of diabetic maculopathy. No animal develops proliferative diabetic retinopathy, for reasons not well understood [18].
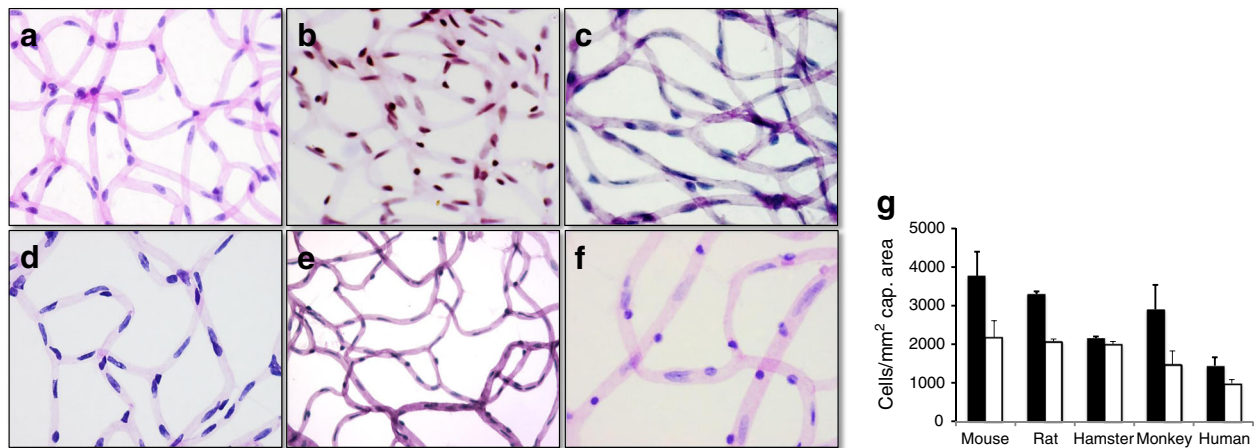
Fig. 2 (**a**–**f**) Digest preparations of mouse (**a**), Chinese hamster (**b**), non-human primate (**c**), rat (**d**), dog (**e**) and human retina (**f**) showing mid-capillary areas. Note the qualitative similarities in cellular compositions. Original magnification, × 400 (**a**–**d**, **f**) or × 200 (**e**). (**g**) Quantitative morphometry of retinal digest preparations of non-diabetic retina, measured as described previously [75]. Data (mean ± SD) show the number of cells per mm^2 capillary (cap.) area. Black bars, endothelial cells; white bars, pericytes

The importance of pericytes

Since the seminal work of Kuwabara and Cogan [19], pericytes have been perceived as the primary trigger of vascular damage in the diabetic retina. Pericytes are enigmatic cells and their diverse ontogeny has prevented a clear structural and functional annotation. Lineage-tracing studies show that most pericytes in the head region (including brain and retina) evolve from the neural crest, some probably derive from mesenchymal stem or progenitor cells, some may expand in situ and some may originate from the bone marrow [20]. This heterogeneity may account for the lack of a universal marker of a retinal pericyte [21]. Pericyte recruitment to the developing retinal vasculature is determined by several ligand–receptor pairs, including the platelet-derived growth factor B–β receptor and the angiopoietin–Tie system [22]. Studies using ablation of one of the recruiting factors or direct pericyte elimination by genetic tools recapitulate early features of experimental diabetic retinopathy such as microaneurysm formation, abnormal blood–retinal barrier (BRB) function, and the formation of acellular capillaries. These studies help underline the functional role of pericytes in maintenance of patent retinal capillaries [23, 24].

The fate of pericytes during physiological vasoregression remains controversial. Potential responses include persistence on the denuded capillary wall, apoptosis and migration onto surviving nearby capillaries [25]. Moreover, signals determining the destiny of the underlying endothelium are not yet understood.

The importance of vasoregression

Vasoregression (the formation of acellular capillaries) is probably the most important early lesion in the early diabetic retina [26, 27]. Understanding the mechanisms that contribute to vasoregression is essential for its prevention, and physiological vasoregression acts as a good example from which we can learn about inborn programming that is reactivated during incipient disease stages. Novel, dynamic, high-resolution microscopic techniques used to study developing rodent vasculature have characterised vessel regression as being: (1) an adaptive response to the exponential growth curve of angiogenesis towards a vascular network matching metabolic and functional demands for maintenance; (2) a process selecting capillary branches for regression by still unknown triggers; and (3) a process involving endothelial cell apoptosis, migration and redeployment as phenotypes [25]. More specifically, the process of vasoregression has been conceptually divided into sequential steps: branch selection by flow dichotomy, vessel constriction, occlusion, endothelial retraction/apoptosis/reintegration and then resolution of the remaining matrix tube. A further distinction between types of vasoregression has been proposed, dependent on the persistence of a patent lumen of the regressive capillary branch (type 1 pruning, closed lumen, resulting from low shear stress/non-perfusion; type 2 pruning, open lumen, high shear stress/perfusion). In this context, blood flow is a critical determinant, since vasoconstriction induces vasoregression while enhanced blood flow protects against vasoregression [28, 29].

Glia: the interface between the vasculature and neurons

The interplay between the microvasculature and the retinal glia during development and in systemic disease such as diabetes has attracted interest [30, 31]. The retina has three glial cell types: microglial cells and two macroglial cell types

(astrocytes and Müller cells). Astrocytes are essential in the incipient formation of the retinal vasculature. Müller cells are specialised radial glia and form the structural and functional link between neurons and blood vessels, handle nutrients and waste products, affect the tightness of the BRB and maintain survival and function of neurons and blood vessels. Several experimental studies show that proper glial function is obligatory for the formation of the deep capillary layers [32–34]. Resident microglia have been described as immunological watchdogs of the retina [30, 35]. They actively sense the microenvironment and contribute to waste removal and defence against degenerative and inflammatory signals. During development, microglia assist retinal remodelling by phagocytosis of dying neurons. Microglia migrate into the retina prior to angiogenesis and influence the different angiogenic steps, demonstrating the complex role of microglial cells as regulators of endothelial sprouting, branch fusion and regression. A recent review provides more details, particularly on new therapeutic strategies for diabetic retinopathy [36].

Modelling early diabetic retinopathy

The clinical course of diabetic retinopathy has been extensively described in several excellent reviews [11, 37]. In animals, it is only possible to model stages which precede lesions that are observed in patients, underlining the need for caution in the translation into clinical studies [38]. Figure 3 summarises the development of experimental diabetic retinopathy established in streptozotocin (STZ)-induced male Wistar rats. Sex, strain and species differences exist between animal models,

explaining some of the reported discrepancies in the presented synopsis. When glucose levels increase, transient breakdown of the BRB can occur within a few days to weeks, associated with transient induction of the paracellular permeability pathway [39]. Intraretinal haemorrhages do not develop in this model. Next, activation of Müller cells, as demonstrated by the expression of glial fibrillary acid protein, ensues after approximately 1.5 months, followed by redistribution of potassium channels and aquaporins at around 4 months [40]. At this point, microglial activation is present, reflected by an increased number of cells as well as altered expression of activation markers. Pericyte loss starts at around month 2 after diabetes onset, due to apoptosis or migration involving the hyperglycaemia-driven angiopoietin–Tie system. As hyperglycaemia continues, endothelial cells are lost, leading to vasoregression after 6 months of diabetes. In experimental models, the prevalent vasoregression phenotype corresponds to the type 1 pruning described above (i.e. a low number of apoptotic cells in relation to the magnitude of vasoregression, and no resolution of empty basement membranes). Migration (and to a lesser extent apoptosis) leads to pericyte loss in remodelling capillaries of animal models but persistence of pericytes on denuded capillary segments is more prevalent in human retinas [26, 41]. Neuronal dysfunction, measured by electroretinography, is evident after 6 weeks, while structural changes indicating neurodegeneration appear only after 6 months [42]. Strain and species differences determine the translational significance, particularly regarding diabetic macular oedema and neurodegeneration. As noted above, there is no consistent appearance of microaneurysms in rodent retinas and retinal oedema or preretinal neovascularisation do not develop in any (rodent) model of diabetes.

Fig. 3 Schematic summary of hyperglycaemia-induced changes of the neurovascular unit in the Wistar rat STZ-induced diabetes model
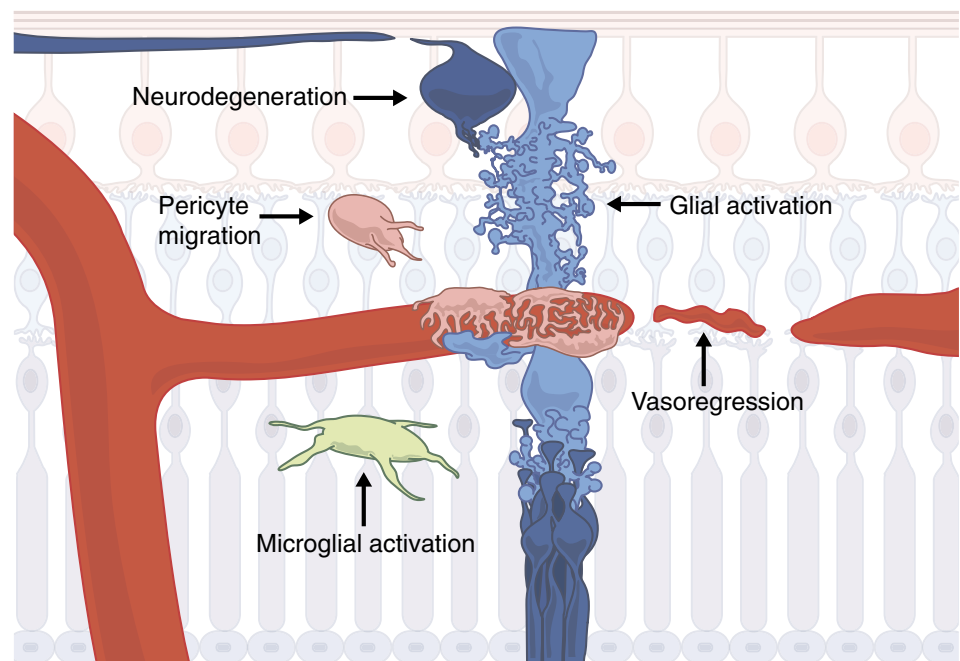
The biochemistry of diabetic retinopathy

Figure 4 summarises some of the known and hypothetical sequences of events in diabetic retinopathy, linking initiating metabolic abnormalities to the functional and structural abnormalities in preclinical models and in humans.

Chronic hyperglycaemia is the major pathogenetic determinant of diabetic retinopathy and most (meta-)analyses highlight disease duration and level of glycaemia (reflected by HbA_{1c}) as being the most important risk factors for disease onset and progression [43–46]. However, the magnitude of their contribution to the risk of complications was put into perspective by the DCCT/Epidemiology of Diabetes Interventions (EDIC) research group, who calculated that HbA_{1c} only accounted for up to 11% of the entire risk for complications and concluded that most of the variation in risk was explained by factors not imaged by glycaemia [47].

The 'unifying hypothesis' [48] may explain how hyperglycaemia can damage the retina: several seemingly independent biochemical pathways overactivated in diabetes are based on one common abnormality, caused by intracellular excess glucose flux, i.e. mitochondrial overproduction of reactive oxygen species [ROS]. The hypothesis proposes that the formation of AGEs can explain the hyperglycaemic memory of tissue damage whereby vascular damage developed during periods of poor glycaemic control persists into cycles of euglycaemia. The reverse

(a delay in worsening under less stringent control after periods of stringent control) has also been shown in the DCCT/EDIC study. The mechanisms of persistent damage through AGEs have been strung into permanent modifications of matrix macromolecules, modifications of intracellular constituents (such as proteins and DNA), altered transcription of proteins and altered cellular functions through receptor-mediated mechanisms. Excess AGE formation and overactivation of the hexosamine pathway induces transcription of angiopoietin-2 by inhibiting a transcriptional co-repressor complex from binding and silencing the angiopoietin-2 promoter [49].

The concept was developed in endothelial cells of different target tissues of diabetes complications and extended to the retina, still with a focus on the diabetic microvasculature, and yielded two pathogenesis-based treatment options. The metabolic signal blocker benfotiamine showed substantial biochemical effects and vascular protection in a rat model of diabetes, even as a secondary intervention approach [50]. Short-term clinical studies have demonstrated benefits on surrogate markers of diabetic nephropathy, but so far there have been no studies on diabetic retinopathy of sufficient duration. The catalytic antioxidant R-α-lipoic acid reduced biochemical and morphological abnormalities in diabetes models, and the combination of benfotiamine and R-α-lipoic acid reduced biochemical pathway anomalies [51].

Fig. 4 Effects of reactive metabolites, transient hyperglycaemia and chronic hyperglycaemia on the neurovascular unit via activation of biochemical and signalling pathways. The resulting neovascularisation, oedema and neurodegeneration represent important advanced clinical stages. CHG, chronic hyperglycaemia; HF, haemodynamic factors; IRMA, intraretinal microvascular abnormalities; MA, microaneurysms; mt ROS, mitochondrial ROS; NVU, neurovascular unit; RM, reactive metabolites; THG, transient hyperglycaemia
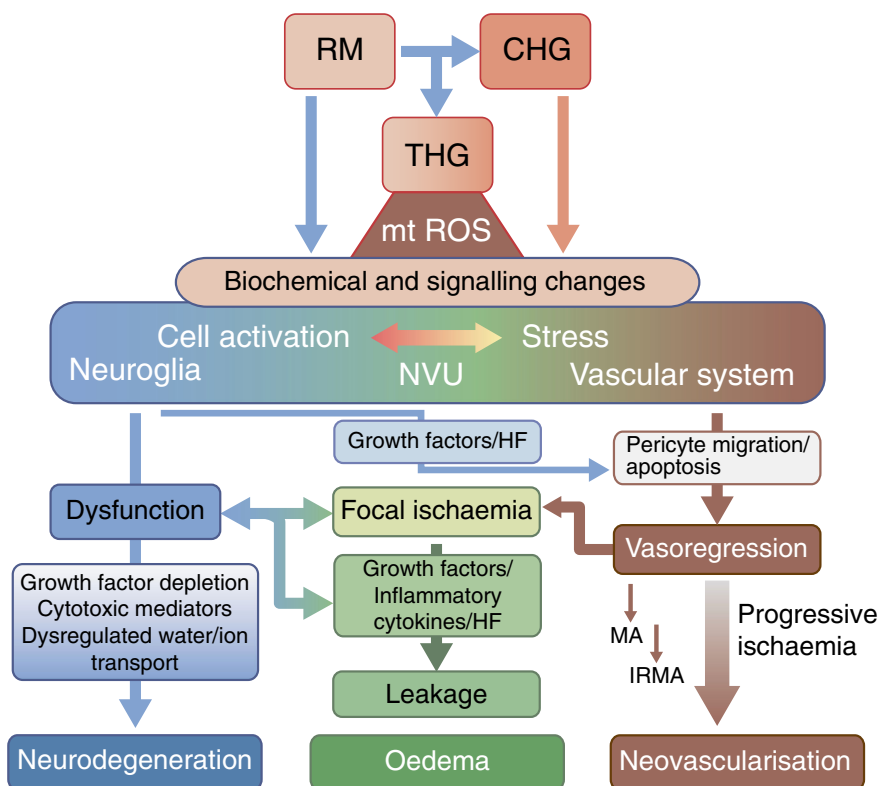
Reactive metabolites in diabetic retinopathy

The unifying hypothesis cannot account for the non-HbA_{1c}-related part of the pathogenesis of diabetic retinopathy. Nawroth and colleagues and Thornalley and colleagues [52, 53] proposed that the role of the AGE precursor methylglyoxal is of profound interest since its formation depends not on hyperglycaemia but on the balance between formation and enzymatic detoxification through the glyoxalase 1 and 2 system. In addition, increased dicarbonyl stress not only arises from hyperglycaemia but also occurs in conditions that promote accelerated ageing and metabolic and vascular complications. Exogenous administration of methylglyoxal to healthy rodents recapitulates diabetic retinopathy-like changes in the neurovascular unit [54], including pericyte loss, formation of acellular capillaries, (micro-)glial activation and early neuronal dysfunction. Gain-of-function studies with Glo-1 reveal its modifier role in the development of retinal damage in the absence of chronic hyperglycaemia [55]. For example, glial activation and vasoregression are attenuated in diabetic rats with a global overexpression of Glo-1. Heterozygous knockdown mice on a C57Bl6J background (45–65% decrease in tissue Glo-1 activity) display vascular accumulation of methylglyoxal hydroimidazolone and increased retinal vasoregression, suggesting that Glo-1 is essential in modifying dicarbonyl stress [56]. As shown in zebrafish, methylglyoxal alters blood vessel formation through altered phosphorylation of VEGF receptor 2 and its downstream target Akt/protein kinase B [57]. In obese individuals, even when glucose levels are still normal and neuroretinal dysfunction is absent, retinal endothelial dysfunction exists in association with minor elevations of plasma methylglyoxal (H.P. Hammes, P. Nawroth, T. Fleming, unpublished observation). Methylglyoxal-scavenging peptides and activators of Glo-1 have been proposed as possible treatments. Recently, a Glo-1-activating combination of resveratrol and hesperetin was reported to produce a meaningful reduction in plasma methylglyoxal and an improvement in endothelial dysfunction in overweight individuals [58]. Experiments addressing the effect of such compounds in diabetic retinopathy are lacking. Indirect evidence that the activation of Glo-1 might improve the neurovascular unit comes from studies using mice deficient in the receptor for AGEs (RAGE). McVicar et al [59] found that Rage (also known as Ager)-knockout mice accumulated less methylglyoxal in their retinas in association with a strong activation of Glo-1 and were protected against microglial activation and vasoregression, with a minor, non-significant effect on pericyte dropout.

Beyond genetic control of Glo-1 activity, established drugs have shown Glo-1-modulating properties. The angiotensin type 1 receptor blocker candesartan normalised Glo-1 activity reduction due to angiotensin II and improved vasoregression and inflammation in retinas of diabetic rats overexpressing the renin–angiotensin system [60].

Targeting the ROS-generating feedback loop and the neurovascular unit

Episodes of transient dysglycaemia occur prior to overt (type 2) diabetes. These episodes are too short to affect HbA_{1c} but are associated with some retinopathy. This scenario has been recently addressed to demonstrate a pathogenetic, multicomponent feedback loop [61]. The loop begins with the release of free iron and superoxide and ends with an impaired association of phosphorylated voltage-dependent anion channel with hexokinase II, shifting the glucose concentration/ROS response relationship to the left. The active glucagon-like peptide-1 (GLP-1) (breakdown) products GLP-1 (7–36)[amide] and GLP-1 (9–36)[amide] are able to reverse the perpetuated ROS production during normoglycaemia and are the first among potential 'loop breakers'. This effect is GLP-1 receptor-independent and, since the retina of rodents lacks the GLP-1 receptor (with the probable exception of the db/db mouse), it is worth noting that several recent studies indicate an improvement in oxidative stress, AGE formation and the neurovascular unit of the experimental diabetic retina in rodents administered with GLP-1 receptor agonists and dipeptidyl-peptidase 4 (DPP-4) inhibitors [42, 62, 63]. To this end, it will be necessary to dissect which part of the preventative effects relates to the action of active GLP-1 as loop breaker and which relate to the GLP-1-receptor-independent mode of action in chronic hyperglycaemia. This is particularly important as in the Trial to Evaluate Cardiovascular and Other Long-term Outcomes With Semaglutide in Subjects With Type 2 Diabetes (SUSTAIN-6) the long-acting GLP-1 receptor agonist semaglutide promoted diabetic retinopathy in the presence of rapidly improved glycaemia, in contrast to an improvement in diabetic nephropathy and cardiovascular outcomes [64]. The effect appeared to be related to a pro-angiogenic effect of semaglutide but the effect of euglycaemic re-entry cannot be excluded. Further analysis of the data and studies in translational adult models of retinal angiogenesis are needed.

The neurovascular unit in the diabetic retina can be targeted using erythropoietin, since all components express the erythropoietin receptor. Erythropoietin has neuroprotective, anti-inflammatory and vasoprotective properties. Suberythropoietic doses of erythropoietin reduced markers of oxidative stress (methylglyoxal-type AGEs), promoted pro-survival signalling and prevented pericyte dropout [65]. In long-term experiments erythropoietin reduced vasoregression and neuroprotection in the diabetic rat retina. Of note, these effects were achieved without normalisation of inflammatory cytokines and prevention of leucostasis [66].

Gasotransmitters in diabetic retinopathy: a mechanism affecting the neurovascular unit

A recent review by van den Born et al describes the role of gasotransmitters in the development of vascular complications in diabetes [67]. Bioavailability of the three gasotransmitters in question, NO, CO and H_2S, is altered in diabetes. These alterations can precede or result from diabetes and their effects on the retina are context dependent.

Inducible NO synthase (iNOS), formed in response to hyperglycaemia, is implicated in the induction of oxidative stress through the reaction of NO with superoxide. iNOS inhibition prevents damage to the diabetic retina and loss of iNOS function in mice provides partial protection against vasoregression and neurodegeneration.

CO is produced by haem oxygenases, which are induced by oxidative stress in the diabetic retina. CO is involved in anti-apoptotic and survival-supporting cell signalling, explaining in part the protection of the diabetic retina by haem oxygenase 1 upregulation through haemin.

H_2S is endogenously produced by two main enzyme systems (cystathionine-β-synthase and cystathionine-γ-lyase) and is known to induce vasodilatation, scavenge ROS and induce angiogenesis. H_2S is reduced in animal models of diabetes. Mice with a heterozygous deletion of cystathionine-β-synthase display a loss of retinal ganglion cells, suggesting that H_2S is neuroprotective. NaHS (an H_2S donor) is vasoprotective in STZ-induced diabetic rats.

Together, the potential of gasotransmitters as protectants for the diabetic retina is suggestive, but needs confirmation.

Oxidative stress in diabetes goes beyond the mechanisms delineated above, as it involves NADPH oxidases (NOX1, 2, and 4) and the key transcription factor NF-E2-related factor 2 (NRF2) which control downstream antioxidant pathways [68, 69]. Possible involvement of epigenetic mechanisms induced by oxidative stress, (para-)inflammatory regulation and the impact of the microbiome on the neurovascular unit are also important, but go beyond the scope of this review.

Models with retinal degeneration: lessons for the diabetic retina

The notion that diabetic retinopathy may have a strong neuroglial component in its pathogenesis has raised interest in animal models in which neurodegeneration is the unequivocal initiator, to better understand the impact of retinal degeneration on the microvasculature. Photoreceptor degeneration in this condition promotes vasoregression. Given the role of the neurovascular unit in disease development and progression, it would be interesting to discover which comes first in the diabetic retina–vasoregression or neuronal degeneration [70]. Although most studies have suggested that vascular changes occur first, recent observations suggest the opposite. Figure 5 summarises the sequence of events in one rat model studied for the components of the neurovascular unit. The model is based on the selective destruction of photoreceptors via insertion of a mutated gene causing malfunction of the cilia. Microarray and ingenuity pathway analyses yielded evidence for involvement of the complement pathway and innate immunity system in the early response to neuronal damage. Novel data combining microarray analysis with laser dissection microscopy of the inner vascularised retina and the outer retina provided further evidence for addition of acute phase proteins and complement components to the list (C. Weinold, M. Kolibabka, G. Molema, H. P. Hammes, unpublished data). In contrast to concepts derived from mice with photoreceptor degeneration, reactive metabolites such as methylglyoxal were present at lower levels in rats with polycystic kidney disease vs control rats, suggesting a different concept behind the link between photoreceptor damage and vasoregression. Induction of diabetes in this model led to an unexpected result: vasoregression was reduced rather than augmented, consistent with previous data in the mouse [36, 71]. The mechanisms underlying this protection may differ between mice and rats: mouse photoreceptors may activate leucocytes to mediate endothelial toxicity, while in the rat, counterregulatory attenuation of vasoregression by small heat shock proteins is conceivable.

Fig. 5 Temporal annotation of changes in the neurovascular unit in a rat model of retinal degeneration in which selective destruction of photoreceptors is achieved via insertion of a mutated gene causing malfunction of the cilia. ERG, electroretinography; PR, photoreceptor
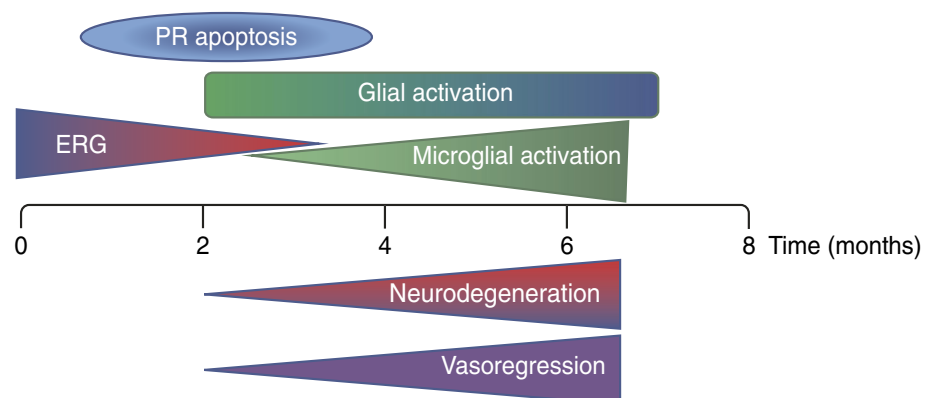
Models with a distinct neuronal onset of retinal damage are clearly discriminable from other animal models of diabetes in the level of primary and secondary compartment destruction and responsive cell activation. Substantial differences in species and kinetics of vasoregression secondary to neurodegeneration exist. Nevertheless, the potential of models enabling curtailment of mechanisms relevant to initiation and propagation of vasoregression in diabetes is eminent.

The kidney and the eye: an 'unhappy alliance'

The coincidence of retinopathy and nephropathy in long-standing diabetes is well-established, and the term 'reno-retinal syndrome' flags the unlucky liaison [39]. Due to the proximity of important structural determinants and overlap of pathways that determine development and ageing, the association of kidney disease with chronic eye disease has been discussed [72]. Genetic determinants such as complement factor H polymorphisms, cardio-metabolic risk factors, oxidative stress and inflammatory signals are thought to explain coincidental eye and kidney pathology. Age, diabetes, hypertension, obesity and smoking further increase the prevalence of chronic kidney disease in adults.

Metabolic and haemodynamic factors, organ-specific growth factors/cytokines and poorly defined genetic and systemic factors cooperate to determine the level and extent of retinal and renal damage. Systemic inflammation induced by the damaged kidney (reflected by increasing severity of albuminuria) is thought to accelerate the clinical course of microvascular complications in both the eye and the kidney [14]. However, clinical proof for the benefit of targeting renal inflammatory signals to reduce the progression of retinal disease to sight-threatening stages is lacking [12].

Outlook

With declining prevalence of diabetic retinopathy in some well-aided areas of the world, and the advent of intravitreal injections of anti-VEGF antibodies or steroids, it has been suggested that the problem of diabetic retinopathy has been solved. However, it should not be forgotten that diabetic retinopathy is part of a systemic disease and predicts cardiovascular morbidity and mortality [73]. The genetics of diabetic retinopathy are clearly distinct from those of diabetic nephropathy and the search for a biomarker for improving the prediction of this malignant clinical course has failed so far, leaving microaneurysms as the best predictor ('biomarker') of the clinical course ('One microaneurysm is not an innocent finding', as E. M. Kohner [Dept. of Medicine, St. Thomas' Hospital, London, UK] has often said).

Maintenance of euglycaemia cannot completely prevent complications of diabetes; it may be difficult to achieve long term, may at some point be deleterious [74] and there is a clear point of no return. Thus, it is clearly important to understand the diabetes-induced changes in the neurovascular unit, develop reliable surrogates for predicting the different clinical courses and stratify the treatment accordingly.

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