Animal Models of Diabetic Retinopathy (Part 1)

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Abstract

Diabetic retinopathy (DR) is one of the leading causes of preventable vision impairment and blindness in the working-age population worldwide. Numerous animal models have been developed for therapeutic drug screening and to further our understanding of the molecular and cellular pathological processes involved in DR. In this book chapter, we describe the cellular, molecular and morphological features of mouse models of DR as well as their respective advantages and limitations. To date, no animal model can holistically reproduce the pathological progression of human DR; most only display early or advanced lesions of DR. However, a thorough understanding of genotypic and phenotypic expressions of existing models will facilitate researchers’ selection of the appropriate model to simulate their desired clinical scenarios.

Keywords: animals, blood glucose, blindness, diabetic complications, diabetes mellitus/pathology/physiopathology, neovascularization, proliferative, retinal vessels

1. Introduction

Diabetes mellitus is a growing epidemic and a major contributor to the global burden of disease [1]. Insulin deficiency leading to hyperglycemia occurs in type 1 diabetes (T1D or insulin-dependent diabetes mellitus) as a result of autoimmune destruction of pancreatic beta islet cells. Type 2 diabetes (T2D or non-insulin-dependent diabetes mellitus) is characterized by insulin resistance, often due to physical inactivity and obesity, and may progress to impaired insulin production. T1D is unpreventable as of current understanding, while T2D, the more common type of the two, is preventable.

Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes and one of the leading causes of preventable vision impairment and blindness in the
working-age population worldwide. It can be broadly classified as non-proliferative diabetic retinopathy (NPDR) or proliferative diabetic retinopathy (PDR). According to the AAO International Clinic DR Disease Severity Scale, NPDR is further subdivided into mild, moderate or severe NPDR, depending on the extent of microaneurysm, intraretinal hemorrhage, venous beading and intraretinal microvascular abnormality (IRMA) formation [2]. With worsening retinal ischemia and increasing microvascular damage, NPDR may progress to PDR, which is characterized by the presence of neovascularization and/or vitreous or preretinal hemorrhage [2]. Severe cases of PDR may result in retinal edema, tractional retinal detachment and neovascular glaucoma. Diabetic maculopathy or macular edema, the most common cause of vision loss, may also arise at any stage of DR [3].

DR-associated visual impairment results in large socioeconomic costs for both the society and individuals. This calls for effective screening methods and increased efforts to understand the pathophysiological progression and to look for effective treatment strategies using both experimental animal models and clinical trials.

2. Pathological features of human diabetic retinopathy

Although DR has long been considered as a hyperglycemia-mediated microangiopathy, it has been recognized as a neurodegenerative process in view of the presence of neurodegenerative abnormalities preceding clinically apparent microvascular changes. Numerous cellular and molecular changes reflective of the DR pathogenesis have been identified, though the multifactorial nature of DR makes it challenging to clearly identify clinically relevant pathogenic pathways implicated in each stage of retinopathy. The common clinical, cellular, molecular features and functional changes of human DR are summarized in Table 1.

2.1. Cellular and molecular features

The DR hallmark lesions of capillary basement membrane (BM) thickening [4, 5] and pericyte loss [6] or apoptosis [7, 8] have been well described in human patients. Other microvascular changes include blood-retinal barrier (BRB) disruption (as evidenced by fluorescein leakage) [9] and the presence of acellular capillaries [6]. In regards to hemodynamics, it has typically been reported that retinal blood flow is increased in NPDR [10–12]. Conversely, in PDR, the nature of retinal blood flow changes appears to be dependent on the degree of non-perfusion and the pathological features present, with no marked increases in blood flow in cases with arterial narrowing [9, 10, 13]. As persistent inflammation is also implicated in DR, studies have demonstrated increased leukostasis (increased leukocyte entrapment and leukocyte endothelial cell adhesion) in diabetic retinae, perhaps resulting from increased expression of adhesion molecules (e.g. ICAM-1) in human DR [14].

Histologically, retinal thinning, particularly thinning of the pericentral total retinal thickness and the retinal nerve fiber layer (RNFL), is present in both TID and T2D patients with no DR, NPDR or pre-proliferative DR [15–19]. Studies analyzing individual intraretinal layer thicknesses showed thinning of the ganglion cell layer (GCL), RNFL, inner plexiform layer (IPL)
and inner nuclear layer (INL) in patients with minimal DR as compared with controls, while such a difference was not observed in diabetic patients without DR [16, 19]. Numerous studies have also documented evidence suggestive of increased retinal ganglion cell (RGC) loss in DR [20].

In addition to neural apoptosis, reactive gliosis is another prominent feature of DR. Expression of glial fibrillary acidic protein (GFAP), an intermediate filament protein expressed by astrocytes, is normally confined to the proximal retina in non-diabetic retinas. In DR, there is aberrant overexpression of GFAP by Müller cells spanning across the entirety of Müller cell processes [21]. Microglial cells are also activated in NPDR [22]. In PDR, the microglia surrounds the neovascularization area in the vitreous, with subsequent infiltration and migration of activated microglia into the subretinal space in cases with diabetic macular degeneration [22].

### 2.2. Electrophysiological alterations

Electroretinographic (ERG) alterations have long been documented in diabetic patients prior to the development of visible lesions of retinopathy. Delay in implicit times of oscillatory features Non-proliferative diabetic retinopathy (NPDR) Proliferative diabetic retinopathy (PDR) (in addition to features of NPDR)

| Features | Non-proliferative diabetic retinopathy (NPDR) | Proliferative diabetic retinopathy (PDR) (in addition to features of NPDR) |
|----------|---------------------------------------------|-------------------------------------------------------------------------|
| Clinical features [2] | • Intraretinal hemorrhages • Microaneurysms • Cotton wool spots • Venous beading • IRMAs (e.g. vessel tortuosity, venous loops, vessel dilatation) | • Neovascularization • Retinal or vitreous hemorrhage • Trabecular retinal detachment (advanced) • Neovascular glaucoma (advanced) • Retinal edema (can occur at any stage of DR) |
| Cellular and molecular features | • RGC loss [20] • Reactive gliosis (overexpression of GFAP expression in Müller cells) [21] • Activated microglia [22] • Decrease in retinal thickness (total, RNFL, GCL, INL, IPL) [15–19] • Pericyte loss [6] or apoptosis [8] • Leukostasis [14] • Capillary BM thickening [4, 5] • Acellular capillaries (associated with microaneurysms) [6] • BRB breakdown [9] • Capillary non-perfusion and obliteration • Increased retinal blood flow [10–12] • Decreased arteriole-to-venule ratio (decreasing with increasing DR severity) [29] | • Retinal blood flow may be increased [11] or equivalent to that of normal patients [9, 10, 13] • Infiltration of activated microglia into subretinal space (diabetic maculopathy) [22] |
| Functional changes (ERG) | • Increased OP peak latencies [25] • Reduced OP amplitudes [23, 25] • Delayed OP implicit times [23–25] • Increased b-wave implicit time [26] • (Reduced b-wave amplitude) [30] | • Reduced b-wave amplitude [25, 27, 28] |

Table 1. Overview of common clinical, cellular, molecular features and functional changes of human DR.
potential (OPs), particularly OP1, precede retinopathy development [23, 24]. The OPs are generated by inner retinal neurons and are often considered to be reflections of feedback circuits between amacrine and bipolar cells and/or circuits between amacrine and ganglion cells. Eyes with NPDR display a reduction in OP amplitudes [24, 25] and an increase in OP peak latencies [25]. There is some discrepancy regarding the onset of changes in b-wave responses, which are largely generated by depolarizing bipolar cells with some contribution from Müller cells. B-wave implicit times appear to be increased even in early stages of DR [26] while reductions in b-wave amplitudes have been suggested to be predominantly found in eyes with PDR [25, 27, 28]. Changes in OP amplitude and implicit times have also been suggested to be a reflection of the severity and prospective progression of DR [24, 25, 27].

3. Models of diabetic retinopathy

Animal models of DR can be broadly classified into (1) diabetic models by pharmacological induction, diet induction or genetic manipulation and (2) non-diabetic models of proliferative retinopathy and angiogenesis. To date, no diabetic models fully develop end-stage retinopathy, arguably due to the short lifespan of animals and differing anatomical structure from humans. Non-diabetic models are thus used to mimic the pathophysiology of end-stage DR, specifically the proliferative pathogenesis and neovascularization in the retinal vasculature. These models, however, are not DR-specific, and display phenotypes common to other conditions with retinal neovascularization. While animal models are useful for drug testing and furthering our understanding of the molecular and cellular pathological processes involved in DR, no single model can holistically reproduce the pathological features of human DR. BRB breakdown, for example, is exhibited in numerous animal models. Yet macular edema resulting from the increase in permeability of retinal capillaries is seldom observed. Judicious evaluation and selection of models according to research objectives is critical to avoid inappropriate translation of experimental findings to the clinical situation. An overview of existing models used to study DR is summarized in Table 2. The cellular, molecular and morphological features of existing animal models of DR are described in Section 4 of this chapter and Section 1 of the following chapter (Animal Models of Diabetic Retinopathy Part 2).

3.1. Diabetic models

3.1.1. Pharmacological induction of diabetes

Pharmacological induction of diabetes is most commonly performed using streptozotocin (STZ), a naturally occurring antibiotic in Streptomyces acromogenes, or alloxan, a pyrimidine derivative. Both chemicals destroy the β-cells of the pancreatic islets. STZ is preferentially used over alloxan due to its greater stability and more preferable chemical properties [31]. T1D or T2D can be induced by varying the dosage and/or number of doses administered, or by combination administration with other treatments (e.g. STZ injection with nicotinamide administration or high fat diet feeding). The use of this model to induce T1D is more common due to the inability of the two chemicals to directly induce insulin resistance. Low doses of
| Model               | Pharmacological induction          | Diabetes | Advantages                        | Limitations                                                   |
|---------------------|-----------------------------------|----------|-----------------------------------|---------------------------------------------------------------|
| Diabetic models     |                                   | Type 1 (or 2) | Quick induction; Lower cost       | Individual animals may demonstrate resistance to STZ-hyperglycemia induction; Requires exogenous injections; Short lifespan of animals; Toxicity of drugs |
| Pharmacological     | STZ-induced; Alloxan-induced       |          |                                   |                                                               |
| induction           |                                   |          |                                   |                                                               |
| Genetically diabetic| Mice: Ins2Akita, NOD mouse         | Type 1 or 2| Consistent phenotype; High success rate of hyperglycemia induction; No further manipulation required | Higher cost; Breeding time required |
|                     | - T1D: Ins2Akita, NOD mouse        |          |                                   |                                                               |
|                     | - T2D: db/db mouse, KKA' mouse     |          |                                   |                                                               |
|                     | Rats:                             |          |                                   |                                                               |
|                     | - T1D: Biobreeding (BB) rat        |          |                                   |                                                               |
|                     | - T2D: Wistar Bonn/Kobori (WBN/Kob) rat, Zucker diabetic fatty (ZDF) rat, Otsuka Long- Evans Tokushima fatty (OLETF) rat, non-obese Goto-Kakizaki (GK) rat, spontaneously diabetic Torii (SDT) rat, TetO rat |          |                                   |                                                               |
| Diet-induced        | Galactose-feeding                  | Type 2   | Longer lifespan of animals; Allows for analysis of retinal features in animals beyond 1 year of age; Isolated elevation of hexose levels without metabolic abnormalities of diabetes | Longer time required to develop DR features |
|                     |                                   |          |                                   |                                                               |
| Model                  | Diabetes | Advantages                                           | Limitations                                                                 |
|------------------------|----------|------------------------------------------------------|------------------------------------------------------------------------------|
| Non-diabetic models    |          |                                                      |                                                                              |
| Oxygen-induced retinopathy (OIR) |          | Continuous hyperoxia → normoxia                      | Consistent and reproducible neovascularization                             |
|                        |          | Alternating cycles of hyperoxia and hypoxia → normoxia |                                                                              |
|                        |          |                                                      | Phenotype not specific to DR                                                |
|                        |          |                                                      | Mostly for small rodents (mice, rats)                                      |
|                        |          |                                                      | Only applicable to newborn rodents                                         |
|                        |          |                                                      | Neovascularization in undifferentiated retina                              |
|                        |          |                                                      | Varying ocular angiogenesis responses in differing strains of rats          |
|                        |          |                                                      | Spontaneous regression of neovascularization features within 1 week of neovascularization development |
|                        |          |                                                      |                                                                              |
| Retinal occlusion      |          |                                                      |                                                                              |
|                        |          | Retinal vein occlusion                              | Neovascularization in fully differentiated retinae                         |
|                        |          |                                                      | Quick induction of neovascularization response                             |
|                        |          |                                                      | Phenotype not specific to DR                                                |
|                        |          |                                                      | Acute ischemia                                                            |
| Intraocular injection  |          |                                                      |                                                                              |
|                        |          | Vascular endothelial growth factor (VEGF)           | Displays NPDR and PDR features (VEGF injection)                           |
|                        |          | (Fibroblast)                                         |                                                                              |
|                        |          |                                                      | Phenotype not specific to DR                                                |
|                        |          |                                                      | Mainly applicable to large animals (e.g. rabbits)                         |
|                        |          |                                                      | Long duration of exogenous injection of pro-angiogenic molecules required  |
|                        |          |                                                      | Mimics proliferative vitreoretinopathy more than ischemic retinopathy (fibroblast injection) |
| Transgenic mice        |          |                                                      |                                                                              |
|                        | Mice: Kimba mice, Akimba mice, TgIGF-I mice         | Exhibits reproducible neovascularization                                  |                                                                              |
|                        | (Akimba: type 1)                                   |                                                      | Cost                                                                         |
|                        |          |                                                      | Some strains not commercially available                                   |
|                        |          |                                                      | Phenotypes may not be specific to DR                                       |
|                        |          |                                                      | Changes do not necessarily occur due to prolonged hyperglycaemia          |

*Table 2.* Overview of existing models used to study DR.
insulin are required for maintenance of STZ or alloxan-induced diabetic animals. It is important to note that failure of hyperglycemia induction may occur in individual animals due to STZ resistance. Blood glucose monitoring is hence essential for confirmation of hyperglycemia development. A review by Lai and Lo [32] comprehensively details existing regimens for induction of diabetes using STZ.

3.1.2. Genetically diabetic animals

Spontaneous hyperglycemia can occur in animals carrying endogenous mutations. Inbreeding of mutated animals with wild-type animals generates reliable hyperglycemic models with consistent phenotype expression. However, the establishment of large colonies may be time-consuming. The target genes for genetic manipulation in specific animal models (e.g. insulin 2 gene mutation in the Ins2\textsuperscript{Akita} mouse; leptin receptor gene mutation in the db/db mouse) are detailed in Section 4 of this chapter and Section 1 of the following chapter.

3.1.3. Diet induced

Experimental galactosemia via feeding with 30–50% galactose can also be used to induce diabetic retinopathy. Galactose feeding causes the isolated elevation of blood aldohexose levels. Other metabolic abnormalities (e.g. alterations in insulin, glucose, fatty acids, amino acid levels) characteristic of diabetes are absent in this model [33]. Despite the long feeding time required for the onset of DR-like lesions, these animals have a longer lifespan than other diabetic models. The model may hence be able to reflect the retinal complications arising from a prolonged period of isolated elevated hexose levels.

3.2. Angiogenesis models

3.2.1. Oxygen-induced retinopathy (OIR) model

Originally developed as a model for retinopathy of prematurity, the oxygen-induced retinopathy (OIR) model has also been used to investigate angiogenesis in other retinal diseases, including proliferative DR. The OIR model is mostly used in small rodents such as mice and rats. In brief, neonatal rodents are exposed to hyperoxia to induce vaso-obliteration. Upon removal from hyperoxia, hypoxia develops in the retina. This triggers a compensatory revascularization response, resulting in neovascularization [34]. This model differs from DR in that OIR-induced neovascularization occurs in incompletely differentiated retinae, while neovascularization in DR results from progressive retinal ischemia and capillary obliteration in fully differentiated retinae.

3.2.1.1. OIR mouse model

The OIR mouse model involves exposing postnatal 7-day-old (P7) mice to 75% oxygen for 5 days before placing them back in normoxia at P12. Upon return to room air, vessel regrowth occurs at P12–P17, with neovascularization beginning at P14. Neovascularization peaks at P17 and complete spontaneous resolution is subsequently achieved by P25 [35, 36].
3.2.1.2. OIR rat model

The OIR rat model involves either continuous hyperoxia or alternating cycles of hyperoxia and hypoxia. In general, the continuous hyperoxia model involves placing rats under 80% oxygen conditions for 22 hours per day until P11. Rats are then transferred to room air for 7 days (P11–P18). In the alternating hyperoxia model, newborn rat pups are exposed to sustained cycles of hyperoxia (50–80%)/hypoxia (SHH) for 14 days and subsequently returned to room air [37, 38]. OIR methods involving the use of varying oxygen concentrations have been described.

3.2.2. Retinal occlusion

Retinal vein occlusion via laser photocoagulation or photodynamic therapy has been used to induce neovascularization in fully differentiated retinae of mice, rats, pigs and monkeys [39–43]. This model induces a near immediate neovascular response with development of retinal edema within hours and the development of intravitreal vessels within days. As DR is predominantly a chronic ischemic disorder, the use of these retinal occlusion models involving periods of reperfusion following acute ischemia induction is less suitable.

3.2.3. Intraocular injection of vascular endothelial growth factor (VEGF)

In view that pro-angiogenic molecules are strongly implicated in retinal neovascularization, researchers have injected VEGF and cultured fibroblasts into monkeys and rabbits, respectively. Intravitreal injection of VEGF in monkeys successfully induced the development of many NPDR and PDR features [44]. However, the rabbit model involving intravitreal injection of fibroblasts mimicked proliferative vitreoretinopathy more than ischemic retinopathy, as the elicited neovascular response was more traumatic and inflammatory than ischemic [45, 46].

3.2.4. Transgenic models

Transgenic mouse models of neovascularization include the Kimba mouse, Akimba mouse and transgenic mouse overexpressing insulin growth factor I, as detailed in the following section.

4. DR features of animal models

Among all of the existing animal models of DR, mice and rats are most commonly used, possibly due to their small size, availability, genetic tractability and relatively faster development of DR lesions as compared with larger animals. Table 3 summarizes the cellular, molecular and morphological features of mouse models of DR. Features of rat and non-rodent models are detailed in the next chapter (Animal Models of Diabetic Retinopathy Part 2).
### Cellular, morphological and vascular features of human DR displayed in mouse models

(Age at which correlates are first reported unless otherwise specified)

**('Time post treatment diabetes, galactosemia, or induction of VEGF overexpression)**

| Mouse model | Type of diabetes onset | Age at onset | NPDR features | PDR features | Functional changes (ERG) |
|-------------|------------------------|--------------|---------------|--------------|--------------------------|
| STZ injection | 1 (or 2) | Within 1 week (wk) | - 7 days*: Müller cell gliosis* [63]  
- RGC loss* [63]  
- 8 days*: increased vascular permeability [109] (2 mo) [52]  
- 2 wks*: increased RGC apoptosis [50]  
- 3–4 wks*: decreased total, GCL, IPL, OPL thickness [51]  
- 4 wks*: decreased arteriolar velocity [58, 59]  
- Decreased venular velocity [58]  
- Decreased arteriolar and venular shear rates [58]  
- Decreased arteriolar and venular blood flow rate [58]  
- Decreased arteriolar and venular diameter (not observed at 8 wks post diabetes induction) [59]  
- 21 days*: increased acellular capillaries* [63] (6 mo) [51, 54]  
- IRMA*s [63]  
- Possible venous dilation or beading* [63]  
- Preretinal neovascular tufts* [63]  
- 5 wks*: reactive gliosis and increased number of astrocytes [47]  
- 6 wks*: reduced number of RGCs [48] (7 wks) [49] (10 wks) [50]  
- 2 mo*: pericyte loss [52] (6 mo)* [53] (9 mo)* [54]  
- Leukostasis [56, 57] (3 mo) [51, 54]  
- 10 wks*: decreased total, INL and ONL thickness [50]  
- 3 mo*: increased number of leukocytes [54]  
- 17 wks*: capillary basal lamina thickening [55]  
- 6 mo*: capillary apoptosis [53] | - 21 days*: neovascularization* [63]  
- 17 wks*: increased density of capillaries suggestive of neovascularization [110]  
*in a novel FOB_FT strain of mice | - 4 wks*: decreased OP3 and total OP amplitude [60, 61]  
Prolonged OP2, 3 implicit time [61]  
- 6 mo*: decreased a-wave and b-wave amplitudes [51] |
| Alloxan injection | 1 (or 2) | 1–4 days [64, 65] | - 7 days*: disorganized capillaries* [63]  
- 21 days*: microaneurysms* [63]  
- IRMA-like lesions* [63]  
- Capillary dilatation with preretinal neovascular lesions* [63]  
- 3 mo*: shortened dendrites in microglia [64]  
*in a novel FOB_FT strain of mice | | - 3 wks*: decreased b-wave amplitude [65, 66]  
- 3 mo*: decreased b/a-wave ratio [64]  
Delayed OPs [64] |
| Mouse model Type of diabetes onset | NPDR features | PDR features | Functional changes (ERG) |
|-----------------------------------|---------------|--------------|-------------------------|
| Galactose-fed / /                | • 11 mo: reduced number of endothelial cells [67] (22 mo) [69] Pericyte loss [67] (22 mo) [69] (26 mo) [33] | • 6–9 mo: retinal neovascularization [73] 7 mo: decreased retinal blood flow rates [79] | • 3 mo: reduced b-wave amplitude 9 mo: Reduced scotopic b-waves [73] Reduced a, b-wave amplitude [77] Increased a, b-wave implicit time [77] Reduced OP amplitude [77] Increased OP implicit time [77] Reduced b/a-wave ratio [77] |
| *Ins2* Akita / 4 wks of age (male mice) | • 13 mo: acellular capillaries [68] (15 mo*) [33] (20 mo) [67] (21 mo) [33, 69] Pericyte loss [67] (22 mo) [69] (26 mo) [33] | • 21 mo: saccular microaneurysms [33] Increased capillary BM thickness [33] | |
|                                   | • 21 mo: saccular microaneurysms [33] Increased capillary BM thickness [33] | *50% galactose diet (remaining = 30% galactose diet) | |
|                                   | • 26 mo: acellular capillaries [68] (22 mo) [69] (26 mo) [33] | | |
|                                   | • 31–36 wks: increased number of acellular capillaries [71] | | |
|                                   | 6–9 mo: microaneurysm formation [73] | | |
|                                   | 9 mo: increased capillary BM thickness [73] | | |
|                                   | *Conflicting results from alternate studies* | | |
|                                   | *In vivo imaging techniques failed to reveal inner retinal thinning [76, 78] | | |
| Mouse model | Type of diabetes | Hyperglycemia onset | Cellular, morphological and vascular features of human DR displayed in mouse models (Age at which correlates are first reported unless otherwise specified) | NPDR features | PDR features | Functional changes (ERG) |
|-------------|------------------|---------------------|-----------------------------------------------------------------------------------------------------------------|----------------|----------------|--------------------------|
| NOD 1       | Female mice: initial onset at 12–14 wks of age [81]; 80% reaching hyperglycemia at 30 wks | • 3 wks: arteriolar vasoconstriction (in close proximity to venules) [83] | Retinal capillary BM thickening [84] Perivascular edema [84] | • 6 mo: retinal microvessel loss [85] Major vessel vasoconstriction or degeneration [85] |
|             |                   | • 4 wks: ganglion cell, pericyte, endothelial cell apoptosis [84] | #changes became more obvious after 12 weeks of hyperglycemia | |
| db/db 2     | 4–8 wks of age [88] | • 8 wks: increased apoptotic cells in GCL [88] (15 mo) [87], INL [89] and GCL [89] Glial activation (increased GFAP expression in Müller cells) [88, 89] (15 mo) [87] ONL thinning [88] DNA fragmentation in photoreceptors [88] | Increased glutamate levels and reduced GLAST content [88] BRB disruption [89] (19 weeks) [111] (15 mo) [87] | • 15 mo: retinal capillary proliferation [87] |
|             |                   | • 16 wks: reduced central and peripheral total retinal thickness [88] | • 18 wks: increased RBC velocity [91] | • 8 wks: Progressive reduced c-wave amplitude [90] Reduction in fast oscillation amplitude [90] |
|             |                   | • 18–20 wks: increased VEGF and decreased PEDF in vitreous [94] | • 22 wks: retinal capillary BM thickening [93] | • 12 wks Reduction in off response amplitude [90] |
|             |                   | • 26 wks: pericyte loss [92] (15 mo) [87] | • 31 wks: increased endothelial cell/pericyte ratio [112] Acellular capillaries [112] (34 wks) [92] | • Increased b-wave implicit time [88] Reduced b-wave amplitude [88, 90] |
|             |                   | • 34 wks: | • 24 wks Reduced a-wave amplitude [90] | • Increased oscillatory potential (OP) implicit time (scotopic conditions) [88] Reduced OP amplitude (scotopic conditions) [88] |
| Mouse model | Type of diabetes | Hyperglycemia onset | Cellular, morphological and vascular features of human DR displayed in mouse models (Age at which correlates are first reported unless otherwise specified) | Time post treatment: diabetes, galactosemia, or induction of VEGF overexpression |
|-------------|------------------|---------------------|--------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| KKA'        | 2                | 5 wks of age [96]   | NPDR features: 3 mo  
- Retinal neuronal cell apoptosis in GCL and medial INL  
- Increased capillary BM thickness [98]  
|             |                  |                     | PDR features: P18 (postnatal day 18) [99]  
- Intravitreal neovascularization across all retinal eccentricities  
- Decreased vessel profiles in deep plexus  
- Absence of vessels in the inner retinal plexus  
|             |                  |                     | Functional changes (ERG): p18 [99]  
- Reduced a-wave, b-wave amplitude  
- Increased b-wave implicit time  
- Reduced OP3, OP4 amplitude |
| OIR         | /                | /                   | NPDR features: P18 [99] Reduced IPL and total retinal thickness  
- Decreased outer segment length  
- Müller cell gliosis (increased GFAP expression)  
- Activated microglia  
|             |                  |                     | PDR features: P18 [99]  
- Intravitreal neovascularization across all retinal eccentricities  
- Decreased vessel profiles in deep plexus  
- Absence of vessels in the inner retinal plexus  
|             |                  |                     | Functional changes (ERG): p18 [99]  
- Reduced a-wave, b-wave amplitude  
- Increased b-wave implicit time  
- Reduced OP3, OP4 amplitude |
| Kimba       | /                | /                   | NPDR features: P7 reduced total, INL, ONL thickness [101]  
- P28 reduced IPL and outer segment thickness [101]  
- Microaneurysms [101, 113] (10 wks) [100]  
- Vascular leakage [101] (moderate phenotypes displaying decline in leakage at 9 weeks and cessation of leakage at 19 wks (mild and moderate phenotypes)) [102]  
- Tortuous vessels [101] (9–19 wks) [102], capillary dropout [101]  
- 6 wks: increased leukocyte adhesion and leucostasis [102]  
- 9 wks: pericyte loss* [102]  
- Acellular capillaries* [102]  
- Reduced vessel length* [102]  
- Reduced area coverage by vessels* [102]  
- Reduced number of crossing points* [102]  
- 10 wks: capillary non-perfusion [100]  
|             | (trVEGF-029)     |                     | PDR features: P28  
- Neovascularization [100]  
|             |                  |                     | Functional changes (ERG):  
*for Kimba mice displaying moderate signs of retinopathy; the observed changes were observed at 24 weeks of age for those with a mild phenotype |
Mouse model | Type of diabetes onset | Hyperglycemia onset | Cellular, morphological and vascular features of human DR displayed in mouse models (Age at which correlates are first reported unless otherwise specified) 
| | | (*Time post treatment: diabetes, galactosemia, or induction of VEGF overexpression)

| Mouse model | | | NPDR features | PDR features | Functional changes (ERG)
|---|---|---|---|---|---
| Akimba | / | / | • 8 wks: uneven retinal thickness on OCT [78]  Pericyte loss [103]  Microaneurysms [78]  Hemorrhage [78]  Vascular leakage (cessation at 20 weeks) [78]  Reduced endothelial junction protein levels [103]  Vessel tortuosity, dilatation, constriction, beading venous loops [78]  Capillary dropout and capillary non-perfusion [78]  Retinal edema [78] | • 8 wks:  • Retinal detachment [78]  • Neovascularization [78] | |
| TgIGF-I | / | / | • 2 mo: pericyte loss [107]  Retinal capillary BM thickening [107]  Acellular capillaries [107]  • 3 mo: Increased GFAP expression in Müller cells and astrocytes [107]  Increased VEGF [107]  • ≥6 mo: venule dilatation [107]  IRMAs [107]  BRB disruption 7.6 mo: reduced ONL and INL thickness [114] | • ≥6 mo:  • Retina and vitreous neovascularization [107]  • Retinal detachment [107]  • Neovascular glaucoma [107] | • 7.5 mo:  • Reduced scotopic b-wave amplitude and oscillatory potential amplitude [114] |
| Intraocular VEGF injection [108] | | | • 2–4 wks*: venous dilatation Microaneurysm  • 8 wks*: vascular leakage *post VEGF injection | • 12 wks*:  • Increase in number of retinal blood vessels in INL |

Table 3. Summary of the cellular, molecular and morphological features displayed in mouse models of DR. This table has been modified from a review by Lai and Lo [32].
4.1. Mouse models

4.1.1. Pharmacological

4.1.1.1. STZ induced

STZ-induced mice are one of the most commonly used DR models for DR characterization and therapeutic drug studies. The mice develop hyperglycemia within 1 week after being injected with a dose of STZ.

STZ-induced mice have been reported to exhibit various NPDR features. Signs of neuronal degeneration, including a decrease in RGC number and reactive gliosis, were observed as early as at 5–6 weeks post-hyperglycemia induction [47–50]. Thinning of the GCL, IPL, OPL and total retinal thickness occurred at 3–4 weeks of hyperglycemia [51], with INL and outer nuclear layer (ONL) thinning by 10 weeks [50]. Microvascular changes included increased vascular permeability within 8 weeks of hyperglycemia, pericyte loss as early as at 2 months [52–54], capillary basal lamina thickening at 17 weeks [55], capillary apoptosis [53] and increased acellular capillaries by 6–9 months [51, 53, 54]. Persistent inflammation resulted in leukostasis at 2–3 months of hyperglycemia [51, 54, 56, 57] with an increased number of leukocytes in the microvasculature at 3 months [54]. Hemodynamic changes have also been documented. There was a decrease in arteriolar and venular velocity, shear rates, blood flow rates and diameter at 4 weeks of hyperglycemia [58, 59]. However, the changes in the arteriolar and venular diameters were no longer apparent at 8 weeks of hyperglycemia and hence may not be a reproducible feature of the model. ERG demonstrated decreased total OP and OP3 amplitudes with prolonged OP2-3 implicit times at 4 weeks of hyperglycemia [60, 61]. One study also noted decreased a- and b-wave amplitudes, though this was not evident in the majority of reports [51].

Evidence regarding diabetes-induced RGC apoptosis and loss remain controversial. Some studies reported increased RGC apoptosis within 2 weeks of diabetes induction [50] and decreased RGC numbers by 6–10 weeks of diabetes [48, 50]. Others found no evidence of RGC apoptosis or GCL cell loss after up to 10 months of hyperglycemia [51, 56, 62]. The transient increase in neural apoptosis and astrocyte activation that regressed after a longer duration of diabetes in one study suggested that such changes may have been induced by STZ toxicity [53]. Variations in the onset of DR features may be attributable to the use of different strains of mice (despite most using C57BL/6 mice) or differing STZ-injection protocols.

More recently, in a study of various inbred strains of mice selected using “The Collaborative Cross” mouse resource, the FOT_FB strain was identified to exhibit a wide range of NPDR and PDR lesions within a significantly shorter duration of hyperglycemia induction. Classical features of neurodegeneration including Müller cell gliosis and RGC loss were displayed 7 days after diabetes induction. Other lesions included IRMAs, dilated vessels resembling venous dilatation and venous beading, increased acellular capillaries, and signs of vessel invasion into the avascular vitreous cavity [63]. The presence of PDR features absent in conventional strains of mice with STZ-induced diabetes may be attributable to the expression of genes implicated in DR in the FOB_FT strain [63]. Though further characterization studies on
this model may be needed, the FOT_FT mouse may represent a novel resource for the study of DR related genes and for testing of therapeutic interventions targeting vascular, neural and inflammation-mediated damage in DR.

4.1.1.2. Alloxan induced

Few studies have examined neuronal and vascular DR features of alloxan-induced diabetic C57BL/6 or albino mice, perhaps due to the absence of demonstrable lesions. About 3 months of alloxan-induced diabetes in C57BL/6 mice failed to induce neuronal apoptosis, glial activation, and microaneurysm and hemorrhage formation [64]. Only functional changes on ERG were observed, with decreased b-wave amplitudes at 3 weeks in albino mice [65, 66] and decreased b/a-wave amplitude ratio and increased OP latency at 3 months of hyperglycemia in C57BL/6 mice [64]. Morphologically, shortened dendrites and thickened proximal processes of microglia suggested the activation of microglia after 3 months of diabetes [64]. In the less conventionally used FOT_FB mouse strain, the study reported disorganized capillaries within 7 days of diabetes induction [63]. By 21 days of diabetes, microaneurysms, IRMAs and capillary dilatation with preretinal neovascular lesions were found in the mice retinae [63].

4.1.2. Diet induced

Mice fed with a 30% galactose diet were found to have reduced endothelial cells and pericyte loss beginning as early as at 11 months of hypergalactosemia [67]. With prolonged hypergalactosemia, the number of acellular capillaries increased [33, 67–69]. By 21–22 months, microvascular changes, including saccular microaneurysms and capillary BM thickening, were present [33]. Variations in age of reported features exist depending on the strain of mice used and the percentage of galactose incorporated into the mice’s diet. The majority of reports used mice on a 30% galactose-fed diet.

4.1.3. Transgenic diabetic mice

4.1.3.1. Ins2Akita mouse

The Ins2Akita mouse is a T1D mouse model carrying an endogenous point mutation in the Mody4 locus (i.e. Insulin 2 gene) with an autosomal dominant mode of inheritance. The mutation results in misfolding of the insulin protein, leading to beta-cell death and decreased insulin secretion, with subsequent development of hypoinsulinemia and hyperglycemia at around 4 weeks of age in male mice. Female mice are less commonly used for DR studies due to their remission to a mild to moderate hyperglycemic state after sexual maturation following transient hyperglycemia during puberty [70]. Males, on the other hand, develop progressive hyperglycemia, resulting in a shortened average survival time of 305 days [70].

Early subclinical DR features in heterozygous Ins2Akita mice retinae have been consistently reported by numerous studies. Cellular changes observed in humans, including increased retinal apoptosis [71–74] and activated microglia, have been documented in mice as early as at 8 weeks of age. RGC loss by 22 weeks has also been evidenced by several groups [71, 72, 75].
Morphologically, there was abnormal swelling in RGC somas, axons and dendrites, with increased dendritic length in ON-type RGCs in three-month old mice [75]. One study revealed increased GFAP expression in Müller cells in 25-week-old mice [76], yet another only found increased GFAP immunoreactivity in astrocytes [71].

Retinal microvascular changes consistent with clinical NPDR have been documented in \textit{Ins}^{2\text{Akita}} mice. It is important to note that advanced DR clinical correlates of proliferative DR, such as preretinal neovascularization, have not yet been detected in this model. Studies have reported increased leucocyte adhesion to retinal vessels in eight-week-old mice [71] with increased retinal vascular permeability [71, 73] and presence of acellular capillaries [71] in older mice. \textit{Ex vivo} and \textit{in vivo} histological analyses demonstrated inner retinal thinning at 22 weeks [71, 74] and 6 months, respectively [77], conceivably due to dopaminergic and cholinergic amacrine cell loss or dendritic atrophy [74]. Total and outer retinal thinning had been evidenced earlier on at 3 months of age [77]. By 9 months, there was increased capillary BM thickness, with evidence of neovascularization and worsening microaneurysm formation [73]. The use of \textit{in vivo} imaging techniques (OCT) in other studies, however, failed to show evidence of retinal thinning [76, 78] and neovascularization (both by histology and \textit{in vivo} imaging techniques) in 25-week-old mice [76]. Vascular function assessments revealed significantly reduced retinal blood flow rates, blood cell velocity and vascular wall shear rates without signs of increased hypoxia in mice after 26 weeks of hyperglycemia [79]. Corresponding functional deficits, as documented by significantly reduced scotopic a-wave, b-wave and OP amplitudes, increased a-wave, b-wave and OP implicit times, and reduced b/a-wave ratio have also been found in mice 9 months of age [73, 77]. It has been suggested that differences in reported DR morphological features may be due to the potential presence of \textit{rd8} mutations in the \textit{Crb1} gene in C57BL/6 N mice used for the generation of \textit{Ins}^{2\text{Akita}} mice. Affected mice have been described to display signs of retinal degeneration and ocular lesions due to the presence of \textit{rd8} unrelated to the mutated genes of transgenic mice [80].

Despite its short average lifespan [70], the \textit{Ins}^{2\text{Akita}} mouse is a well-characterized model of T1D exhibiting changes associated with early DR. It’s stable insulin-deficient diabetic state that does not require exogenous administration of insulin and lack of systemic immunologic modifications makes it ideal for DR therapy testing. However, it still fails to display preretinal neovascularization and other features of advanced-stage DR.

4.1.3.2. Non-obese diabetic (NOD) mouse

The Non-obese diabetic (NOD) mouse spontaneously develops T1D beginning from 12 to 30 weeks of age. An autoimmune process involving CD4\(^+\) and CD8\(^+\) cells triggers insulinitis and subsequent overt T1D in 80% of female and 20% of male mice by the age of 30 weeks [81, 82].

After 3 weeks of hyperglycemia, constriction of retinal arterioles in close proximity to venules was observed in NOD mice [83]. There was evident degeneration of RGCs, endothelial cell and pericyte apoptosis, retinal capillary BM thickening, perivascular edema and microvascular occlusion by 12 weeks of hyperglycemia (pathological changes initially arose after 4 weeks of hyperglycemia) [84]. Six-month-old mice exhibited further vascular changes, including retinal microvessel loss, vasoconstriction or degeneration of major vessels and focal proliferation of new vessels [85].
Only female mice were used in the studies due to the inconsistent and low rates of hyperglycemic induction in males. However, estrogen is speculated to play a protective role in DR. This may arguably affect the interpretation of potential therapeutic drug studies [32]. Although the NOD mouse represents an autoimmune diabetic model similar to the pathogenesis of human T1D, the onset of hyperglycemia is highly variable, making it a less reliable model for DR studies.

4.1.3.3. Db/db mouse

The C57BL/KsJ-db/db or Lepr<sup>db/db</sup> (db/db) mouse is a T2D model carrying a mutation of recessive inheritance in the leptin receptor gene. Homozygotes develop obesity at 3–4 weeks of age, and hyperglycemia at 4–8 weeks [86].

The mice exhibited progressive neuronal cell loss [87], glial activation [87], neuroretinal thinning, BRB disruption and accumulating glutamate concentrations accompanied with downregulation of the glutamate/aspartate transporter (GLAST) as early as at 8 weeks of age [88, 89]. Progressively worsening retinal function and retinal pigment epithelium dysfunction with persistent hyperglycemia have been evidenced by ERG changes (a-wave, b-wave, c-wave and oscillatory potential changes) beginning at 8 or 16 weeks of age [88, 90]. Sustained hyperglycemia is also suggested to be associated with increased RBC velocity in these mice at the age of 18 weeks [91], though the nature of microcirculatory hemodynamic changes in diabetes remains controversial. Upon lowering of blood glucose levels by dietary restriction, many of the observed neurodegeneration abnormalities regressed or were arrested [88]. Such findings suggest that the observed neurodegeneration features are attributable to the effect of diabetes as opposed to genetic factors.

Microvascular complications, such as pericyte loss [87, 92], presence of acellular capillaries [92] and thickening of the capillary BM [93], were also displayed in this model. Retinal angiogenesis dysregulation in these mice is further supported by corresponding associated biochemical changes in the vitreous and retina associated with DR pathogenesis (increased VEGF and decreased pigment epithelium-derived factor (PEDF)) [94, 95]. The presence of more advanced features of DR, however, is limited to the proliferation of retinal capillaries at 15 months of age [87].

While the model confers signs of retinal neurodegeneration, the mice have a shortened life span and do not breed well [86]. Homozygote females are infertile and homozygote males have low fecundity. Despite such limitations, with numerous reports characterizing structural abnormalities and increasing studies examining its functional deficits in recent years, the db/db mouse remains an extensively used model for therapeutic drug research.

4.1.3.4. KKA<sup>y</sup> mouse

The KKA<sup>y</sup> mouse (or Yellow KK mouse) is a congenic strain of the KK mouse. It was created through the transfer of the yellow obese gene (A<sup>y</sup>) into KK mice, on the basis that diabetic traits were inherited by polygenes [96]. The mice develop hyperglycemia, hyperinsulinemia and obesity beginning at around 5 weeks of age and display marked hyperglycemia by 16 weeks of age [96]. At the age of 40 weeks, the mouse reverts back to normal [97]. Only one
study to date has documented retinal changes in the KKAy mouse. The study reported retinal neuronal cell apoptosis in the GCL and inner INL [98] with capillary BM thickening [32] after 3 months of hyperglycemia.

4.1.4. Angiogenesis models

4.1.4.1. Oxygen-induced retinopathy (OIR)

Characterization of retinal features exhibited by mouse models of OIR has been performed on postnatal day 18-old (P18) mice [99]. Documented cellular features included reduced IPL, outer segment (central and mid-peripheral) and total (central) retinal thickness, and increased gliotic Müller cells and reactive microglia predominantly in areas where deep plexus vascularization was absent. Substantial intravitreal angiogenesis was present in all retinal eccentricities. The number of vessels was reduced in the inner and deep vascular plexuses (central and mid-peripheral), with the central retina remaining fairly avascular. Corresponding functional changes on the ERG were also observed. A-wave, b-wave, OP3 and OP4 amplitudes were reduced and the b-wave implicit time was increased. The OIR model is not widely utilized for therapeutic drug studies for DR, owing to the spontaneous regression of neovascularization within a week of its development.

4.1.4.2. Kimba mouse

The Kimba trVEGF029 mouse (Kimba) is a neovascularization model whereby photoreceptor-specific human VEGF$_{165}$ overexpression is induced using a truncated rhodopsin promoter [100]. The Kimba mouse line displays features most similar to NPDR or early PDR out of the four hVEGF-overexpressing transgenic mouse lines generated, while displaying stable mild to moderate retinopathy for at least 3 months [100]. The phenotypic observations discussed below correspond to the Kimba trVEGF029 individuals displaying mild or moderate retinopathy.

Vascular changes in this model have been documented as early as at postnatal day 7 (P7), with INL, ONL and total retinal thinning as one of the first features displayed. P28 mice exhibited classical features of NPDR (tortuous vessels, microaneurysms, vascular leakage and capillary hemorrhages) that progressed with increasing age [100–102]. The development of such retinal vascular abnormalities was accompanied by increasing adherent leucocyte numbers corresponding to the severity of the abnormality observed [102]. Counter intuitively, vascular leakage began to cease at 9 weeks among moderate phenotypes, but this is most likely due to the significant reduction in hVEGF$_{165}$ expression. Mild neovascularization and altered retinal vasculature demonstrating reduced vessel length, coverage area and crossing points have also been reported in mice 9 weeks of age [102]. However, the observed neovascular changes in such VEGF models occur in the outer retina, as opposed to the inner retina as seen in DR. While new vessels typically grow into the vitreous in DR, vessel growth in this model occurs in the opposite direction, from the capillary bed to the ONL. There has not been widespread use of the Kimba mouse in DR studies perhaps as a result of the commercial unavailability of the mouse.
4.1.4.3. Akimba mouse

To create a hyperglycemic model displaying signs of PDR, the \textit{Ins2}^{Akita} mouse was crossbred with the Kimba mouse to generate the Akimba mouse (\textit{Ins2}^{Akita}VEGF$^{+/−}$). With the inheritance of diabetic and retinal neovascular phenotypes from parental strains, the Akimba mouse exhibits most characteristic features of NDPR and PDR, with the exception of preretinal neovascularization. By 8 weeks of age, the Akimba mouse had developed major retinal microvascular abnormalities including vessel tortuosity, venous loups, vessel beading, vascular dilatation, microaneurysms and non-perfused capillaries [78]. Increased vascular leakage was accompanied with lowered levels of endothelial junction proteins [103]. Significant capillary drop out resulted in leakage cessation at 20 weeks of age [78]. Neural retinal thickness decreased with age [78]. Severe loss of ganglion cells and complete photoreceptor loss occurred in 24-week-old mice [78]. Retinal edema, neovascularization and retinal detachment were also present in the mice at an early age. The vascular changes observed here were more severe than those of Kimba mice [78], suggestive of the dual (and possibly synergistic) effects of simultaneous hyperglycemia and VEGF upregulation, and the potential use of this model to study the interaction of these two factors in DR. However, the vascular abnormalities may have developed predominantly due to VEGF upregulation rather than longstanding hyperglycemia as seen in human DR, making the model unsuitable for etiological studies. In spite of such dissimilarities in the sequential pathogenic processes, the Akimba mouse is a unique model simulating an advanced human DR retinal environment.

4.1.4.4. TgIGF-I mouse

Insulin-like growth factor I (IGF-I) is a VEGF inducer that has been associated with the pathogenesis of DR. Clinically, increased levels of IGF-I have been found in the vitreous of DR patients [104, 105]. To create a model of neovascularization via increased VEGF expression, the RIP/IGF-I chimeric gene was first introduced into mice with a C57BL/6-SJL background, and these mice were subsequently backcrossed to CD-1 mice to create transgenic mice overexpressing insulin-like growth factor I (TgIGF-I) [106]. The mice were reported to exhibit NPDR-like features at the age of 2 months, including pericyte loss, retinal capillary BM thickening and presence of acellular capillaries [107]. With increasing age, there was progressive development of venule dilatation, IRMAs, retinal and vitreous neovascularization, and subsequent retinal detachment [107]. The model has also been found to induce rubeosis iridis, neovascular glaucoma and cataract under normoglycemic and normoinsulinemic conditions [107].

4.1.4.5. Intraocular injection of VEGF

As intraocular injections of VEGF are less feasible in rodent models, subretinal injection of a binary recombinant adeno-associated virus construct producing green fluorescent protein (GFP) and VEGF was used in one study. VEGF overexpression resulted in microaneurysm formation, venous dilatation and vascular leakage [108]. However, the model failed to induce the pronounced neovascularization seen in transgenic animals and was only able to manifest
some features of NPDR. Significant new vessel formation was restricted to the INL of VEGF expression site. Only one mouse displayed signs of retinal degeneration with blood vessel growth into the subretinal space.

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