Therapies for hyperglycaemia-induced diabetic complications: from animal models to clinical trials

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Abstract | Long-term diabetes increases the likelihood of developing secondary damage to numerous systems, and these complications represent a substantial cause of morbidity and mortality. Establishing the causes of diabetes remains the key step towards eradicating the disease, but the prevention and amelioration of diabetic complications is equally important for the millions of individuals who already have the disease or are likely to develop it before prophylaxis or a cure become routinely available. In this Review, we focus on four common complications of diabetes, discuss the range of pathologies that are precipitated by hyperglycaemia and highlight emerging targets for therapeutic intervention.

The increasing prevalence of diabetes, particularly among teenagers §, reinforces concerns over the appearance of the complications of long-term diabetes during what should be the most active and productive years of life. Patients with type 1 (insulin-dependent) or type 2 (insulin-independent) diabetes develop secondary complications, the risk of which is related to the duration of diabetes and the degree of glycaemic control †. The organs that are susceptible to diabetic complications exhibit insulin-independent glucose uptake and possess the glucose-metabolizing enzyme aldose reductase. Therefore, initial interest focused on hyperglycaemia and the subsequent increased glucose flux that is mediated by aldose reductase and the rest of the polyol pathway as a primary pathogenic insult that initiates diabetic complications §.

Unfortunately, almost 40 years of clinical investigation have been unable to show the efficacy of aldose reductase inhibitors to the satisfaction of European and American regulatory bodies. The search for sites of therapeutic intervention has therefore extended to the downstream effects of aldose reductase activity and also to aldose reductase-independent mechanisms of glucose toxicity. Glucose-independent mechanisms of organ damage that arise from other physiological consequences of diabetes, such as impaired insulin and growth factor signalling, hyperlipidaemia and hypertension, could also be instigators of, or contributors to, the aetiology of specific diabetic complications. This mixture of universal and organ-specific mechanisms is reflected in the diverse range of therapeutic approaches that are currently being investigated (FIG. 1).

The four most common complications of diabetes — macrovascular disease, nephropathy, retinopathy and neuropathy — share numerous mechanisms by which hyperglycaemia can disrupt cell and organ function (FIG. 2), with vascular dysfunction also affecting the kidneys, eyes and nervous system. In this Review, we discuss recent developments in preclinical and clinical research for each of these complications to illustrate therapeutic approaches that target either the full range of diabetic complications or the damage to individual organs.

Diabetes and macrovascular complications

Both type 1 (REFS 4,5) and type 2 (REFS 6–8) diabetes have a largely irreversible and devastating effect on small and large blood vessels, and the consequences of vascular injury, such as hypertension, altered vascular permeability and ischaemia, can also contribute to the other complications of diabetes. Heightened oxidative stress and inflammation are increasingly recognized as being central pathogenic mechanisms and markedly alter patterns of gene expression in the vasculature §, shifting the balance from anti-inflammatory and anti-thrombotic homeostatic set points towards an increased pro-inflammatory and thrombogenic potential. As diabetes is further burdened by failure of vascular repair ‖, complications in many organs cause substantial morbidity and early mortality, with heart attack or stroke being the leading causes of mortality in both type 1 (REF 4) and type 2 (REF 13) diabetes.

Animal models and cardiovascular disease. Assorted animal models have been used to dissect the mechanisms...
underlying cardiovascular complications of diabetes and to identify possible therapeutic targets. Macrovascular complications have been studied using both rodent and larger animal models, such as rabbits, dogs, pigs and non-human primates; each species has its advantages and pitfalls. For example, although diabetes enhances vascular permeability in rodents, advanced atherosclerosis is absent in this model, largely owing to the highly effective lipid-clearance mechanisms that are found in these species. Rodent models that combine hyperglycaemia with dietary modulation or genetic modifications to induce hyperlipidaemia and promote atherosclerosis have therefore been developed. However, a consequence of combined hyperglycaemia and hyperlipidaemia in some of the well-studied mouse models, such as apolipoprotein E-deficient mice with concurrent type 1 diabetes, is that cholesterol levels are higher in diabetic animals compared with non-diabetic animals. This makes it difficult to assess the relative contributions of glucose and/or lipids in driving the acceleration of atherosclerosis.

Two particularly creative models have been developed to address this issue. First, to assess the relative roles of hyperglycaemia and dyslipidaemia, mice lacking the low density lipoprotein receptor (LDLR) were crossed with transgenic mice expressing a viral protein under the control of the insulin promoter. When infected with the virus, the mice developed type 1 diabetes. In diabetic animals that were kept on a cholesterol-free diet, atherosclerosis was accelerated compared with non-diabetic animals, suggesting that hyperglycaemia drove accelerated atherosclerosis. However, when the mice were fed diets that were high in cholesterol (which rendered them diabetic), they developed severe hypertriglyceridaemia and advanced atherosclerosis. These studies suggest that diabetes-associated dyslipidaemia accelerated lesion progression. Recent studies using this model have also shown that induction of type 1 diabetes in LDLR-deficient mice promoted plaque disruption, as measured by intraplaque haemorrhage. Interestingly, plaque disruption was associated with the accumulation of monocytes that expressed S100 calcium-binding protein A9, a marker of inflammation.

The second model explores the role of the polyol pathway in the vascular complications of diabetes in mice, a species which normally has low levels of aldose reductase, by amplifying aldose reductase expression to physiologically relevant human levels. These aldose reductase-transgenic mice showed enhanced vulnerability to hyperglycaemia-induced atherosclerosis and ischaemia-reperfusion injury. Importantly, hyperglycaemia seemed to be the primary factor driving accelerated atherosclerosis in aldose reductase-transgenic mice that were also deficient in LDLR, as lipid levels were the same in diabetic transgenic and wild-type animals. These and other animal models are helping to dissect the contribution of different pathogenic mechanisms to diabetic vascular damage.

The paucity of models for diabetic macrovascular complications has prompted the formation of the Animal Models of Diabetes Complications Consortium (AMDCC) to develop and evaluate new models. However, although mouse models are suitable for dissecting and validating the specific role of hyperglycaemia in the pathogenesis of accelerated atherosclerosis in diabetes, they have definite shortcomings. For example, despite developing extensive and highly advanced atherosclerosis and intraplaque haemorrhage, current diabetic mouse models fail to reliably display evidence of thrombosis and overt myocardial infarction. By contrast, hyperglycaemic and hyperlipidaemic pigs and non-human primates with long-standing diabetes can have highly advanced lesions that are more similar to those observed in human subjects and, importantly, that might be vulnerable to thrombosis.

One key component for accurate modelling may be the duration of diabetes, which may require many months to years to generate overt pathology. This has prompted extensive efforts to identify biomarkers of vascular injury in diabetes. Endothelial cell dysfunction, manifested as impaired vasodilatory responses to acetylcholine or reduced blood flow in human subjects, could prove useful in assessing the state of disease and, possibly, the effect of therapeutic intervention on vascular stress. Studies that illustrate the potential diagnostic and predictive value of endothelial cell biopsy techniques in diabetes may also hold promise as an easily repeatable means to sample the vasculature and assess its inflammatory and thrombotic potential at any time point.
Despite the limitations of the various animal models, these studies have shed light on some of the fundamental mechanisms that contribute to accelerated atherosclerosis in diabetes. When testing potential therapeutic targets, smaller animals could be used to probe mechanisms and to carry out early compound screening with a higher throughput, and larger animals could be subsequently used for the final testing of lead compounds and the validation of the mechanisms that were delineated in the smaller rodent species.

Mechanisms and interventions derived from animal models. The key to discovering treatments that target cardiovascular disease in individuals with diabetes lies in identifying the molecular species that promote perturbation of the vessel wall. Extensive epidemiological evidence suggests that glucose is one of the key players in this process. Both the direct and indirect consequences of increased blood glucose levels contribute to the pathogenesis of accelerated cardiovascular disease in diabetes. In the macrovasculature, high levels of glucose probably synergize with superimposed stresses that are common to both diabetes and non-diabetic vascular disease, such as raised levels of serum lipids, hypertension and the sequelae of innate ageing processes, to continuously stress vascular cells. Such mechanisms lead not only to primary vascular dysfunction, but also to chronic cycles of stress that injure surrounding cells, which are dependent on intact vascular function. As summarized in Fig. 2, excess glucose directly stimulates activation of the polyol pathway and also the activity of mitochondria, protein kinase C (PKC) and NADPH oxidase, which results in the production of reactive oxygen species (ROS). Hyperglycaemia also leads to the formation of advanced glycation end products (AGE), which irrevocably alter the diabetic vasculature, leading to vascular stiffening owing to extensive protein cross-linking. Moreover, extracellular AGE also bind and activate the signal transduction receptor RAGE (receptor of AGE). RAGE is a multi-ligand receptor, and its interactions with AGE and non-AGE pro-inflammatory ligands, such as S100–calgranulins and high-mobility group box 1 protein (HMGB1), are potent generators of accelerated vascular inflammation. The importance of these pathways has been further suggested by recent studies involving the generation of unique mouse models. For example, the role of RAGE has been examined in both non-diabetic and diabetic mice deficient for the genes that encode apolipoprotein E and RAGE, and these studies have shown that RAGE has a pivotal role in atherosclerosis.

Clinical development of therapeutics. The insights gained from experimental models such as those described above have led to clinical trials investigating antagonism of the polyol pathway, PKC isoforms (particularly PKCβ), AGE and agents that counter the effect of the enhanced formation of advanced glycation end products (AGE), which irreversibly alter the diabetic vasculature, leading to vascular stiffening owing to extensive protein cross-linking. Moreover, extracellular AGE also bind and activate the signal transduction receptor RAGE (receptor of AGE). RAGE is a multi-ligand receptor, and its interactions with AGE and non-AGE pro-inflammatory ligands, such as S100–calgranulins and high-mobility group box 1 protein (HMGB1), are potent generators of accelerated vascular inflammation. The importance of these pathways has been further suggested by recent studies involving the generation of unique mouse models. For example, the role of RAGE has been examined in both non-diabetic and diabetic mice deficient for the genes that encode apolipoprotein E and RAGE, and these studies have shown that RAGE has a pivotal role in atherosclerosis.
Atherosclerosis
A chronic inflammatory response in the wall of large blood vessels during which the vessel wall hardens owing to accumulation of plaques that are formed following oxidation of low density lipoproteins in the blood by reactive oxygen species.

Endothelial cell
Simple squamous epithelial cell that lines the lumen of all blood vessels and is considered an important site of diabetes-induced damage to blood vessels and subsequently other organs.

Protein kinase C
A family of enzymes that, when activated, translocate to the plasma membrane and facilitate phosphorylation of other proteins, thereby activating or deactivating them. Aberrant activation of PKC has been implicated in the pathogenesis of many diabetic complications.

NADPH oxidase
A membrane-bound enzyme complex that uses NADPH to catalyse the conversion of oxygen to superoxide and thereby generates reactive oxygen species. Widely studied as a mechanism of cell-mediated bacterial killing, excessive activity of NADPH oxidase isoforms has also been implicated in mechanisms of diabetic complications.

Advanced glycation end product
A product of the irreversible addition of glucose to proteins or fats. It is produced following a sequence of Amadori, Schiff and Maillard reactions, which can change the function of the recipient molecule.

Proteinuria
The appearance of serum proteins (for example, albumin) in the urine that is associated with early stages of diabetes-induced damage to the filtration system of the kidney.

Vasculopathy
Damage to blood vessels. Usually divided into damage to large (macrovascular disease) and small (microvascular disease) blood vessels.

and sites in the vessel wall, many mechanisms synergize to cause diabetic vascular stress. Whether suppression of these maladaptive messengers in the vessel wall will also facilitate the recruitment of progenitors and mediate vessel repair is a largely unexplored question. It is plausible that mechanisms which directly injure the vessel wall also disrupt the environmental cues and the flow of repair species and cells to the injured vessel. The long-term safety and tolerability of RAGE antagonism should be more fully investigated, particularly as studies in acute peripheral nerve injury suggest reparative roles for RAGE in inflammatory and axonal element signalling.

As new targets and concepts are investigated, it is important to note that inflammation and adaptive immune mechanisms that contribute to the pathogenesis of type 1 and type 2 diabetes also play a part in the subsequent macrovascular complications. In this context, strategies that broadly suppress inflammation, such as HMGB–CoA reductase inhibitors (statins) and peroxisome proliferator-activated receptor (PPAR) agonists, have been considered relevant to the treatment of diabetic vascular injury. Statins are now considered almost routine in the management of both type 1 and type 2 diabetes, as various studies testing a range of these agents have shown a reduction in cardiovascular morbidity and mortality.

The case for PPAR agonists is less clear, despite good efficacy in experimental models of diabetes-associated atherosclerosis. A recent study that evaluated the PPARγ agonist rosiglitazone using intravascular ultrasound suggests a trend towards reduced progression of atherosclerotic events in a cohort of subjects with type 2 diabetes, although this drug has also been associated with increased cardiovascular events. By contrast, another PPARγ agonist, pioglitazone, showed modest clinical benefits in a study on subjects with type 2 diabetes. Dual PPARα–PPARγ agonists have been developed as a therapeutic approach to improved glucose and lipid homeostasis in patients with diabetes. However, fenofibrate did not clearly show benefits on cardiovascular outcomes despite a reduction in diabetic microvascular disease, and muraglitazar was withdrawn because of possible increased cardiovascular events.

Although vascular inflammation may be the final common mediator and manifestation of diabetic vascular stress, there is little doubt that inhibiting these harmful pathways at earlier stages of hyperglycaemia is fundamental to preventing the devastating effects of diabetes on blood vessels. The importance of ambient hyperglycaemia per se in macrovascular disease may have to be reconsidered in the context of findings from two recent clinical trials, which explored the effects of tight glycaemic control in subjects with type 2 diabetes and established cardiovascular disease. However, there are potential caveats to these studies. The lack of a positive effect on cardiovascular mortality in these studies could be related to the short duration of the trials (<5 years) or poor tolerance of the demanding and extensive therapeutic regimens by older subjects with type 2 diabetes and its many complications. It is also possible that irreversible vascular changes, such as advanced glycation or ‘hyperglycaemic memory’ resulting from previous periods of hyperglycaemia, could also contribute, possibly through epigenetic mechanisms such as glucose-induced histone modifications that alter vascular gene expression. As the effect of diabetes on plasma lipids and blood pressure could also have a substantial role in atherosclerosis, a therapeutic regimen that incorporates antihypertensive and lipid-lowering agents along with drugs that target the consequences of hyperglycaemia may ultimately prove to be the most effective approach, as recently highlighted in the follow-up of a study trialling this approach.

Diabetic nephropathy
Diabetic nephropathy is now the most common cause of end-stage renal failure in the Western world. From a clinical perspective, it is characterized by the onset of proteinuria and a subsequent decline in glomerular filtration rate and ultimate progression to uraemia, which is fatal if left untreated. The main clinical associations that frequently precede overt diabetic nephropathy are hypertension and poor glycaemic control. Once nephropathy is established, blood pressure often rises further, but glycaemic control can paradoxically improve as a result of reduced renal insulin clearance. Both glucose-dependent pathways that are common to vasculopathy and other complications of diabetes, and more organ-specific mechanisms that are linked to systemic and intraglomerular hypertension, seem to play important parts in the development and progression of this disease.

The drugs currently used to treat diabetic renal disease largely target the hypertensive component. In particular, drugs that interrupt the renin–angiotensin system (RAS), such as angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor (AT2) antagonists, are currently considered first-line treatments for diabetic nephropathy, and this therapeutic strategy is incorporated into most national and international treatment guidelines. This pharmacological approach has been extended by the recent production of the renin inhibitor aliskerin, which also seems to reduce proteinuria, particularly as part of dual therapy with an AT2 antagonist. The most effective approach to blocking the RAS remains controversial, and various strategies are currently being investigated. With greater understanding of the complexity of the RAS and identification of new components of this pathway, such as ACE2, it has become increasingly evident that there is a complex interaction between the vasoconstrictor and vasodilator arms of the RAS. Indeed, it seems that an alteration in one component, such as ACE2, can influence the renal response to agents such as ACE inhibitors. Furthermore, there is often upregulation of upstream components such as renin with distal interruption of the RAS, which can be suppressed by vitamin D administration.

Animal models of diabetic nephropathy. The classical model of streptozotocin-induced type 1 diabetes in rodents is still widely used and has a range of functional and structural changes that are comparable to human diabetic
Table 1 | Selected therapies in development for treating diabetic complications

| Drug name       | Drug type                        | Presumed mechanism of action                           | Clinical trial phase |
|-----------------|----------------------------------|-------------------------------------------------------|---------------------|
| Omacor*         | n-3 fatty acids                  | Enhances endothelial cell function and reduces vascular inflammation | IV                  |
| POMx*           | Antioxidant derived from pomegranate juice | Reduces oxidative stress                                | IV                  |
| Rosiglitazone*  | PPARγ agonist                    | Reduces insulin resistance and inhibits inflammatory cytokines | III and IV          |
| Aliskiren*†     | Renin inhibitor                  | Reduces hypertension                                   | III (for angiopathy); III and IV (for nephropathy) |
| Alagebrium†     | AGE cross-link breaker           | Reduces AGE accumulation                                | II                  |
| TTP488§         | RAGE antagonist                  | Reduced RAGE signalling                                 | II                  |
| Pirfenidone§    | Anti-fibrotic                    | Reduces interstitial fibrosis and glomerulosclerosis   | II                  |
| Bevacizumab, ranibizumab and macugen§ | Anti-VEGF agents                  | Inhibits VEGF signalling, angiogenesis and vascular permeability | I–III               |
| Candesartan‡    | Angiotensin II inhibitor and antihypertensive agent | Inhibits angiogenesis and vascular permeability       | III–IV              |
| Infliximab‡     | Monoclonal antibody against TNF  | Inhibits TNF signalling                                 | III                 |
| Doxycycline§    | Antibiotic                       | Inhibits inflammation                                  | II                  |
| Triamcinolone§  | Corticosteroid                   | Inhibits inflammation and vascular permeability        | I and II            |
| Allopurinol, α-lipoic acid and nicotinamide§ | Antioxidant cocktail              | Reduces oxidative stress, inhibits PARP and increases blood flow | III                 |
| C-peptide§      | Fragment of pro-insulin          | Enhances insulin signalling or neurotrophic support    | II                  |
| SB-509§         | Zinc finger protein activator    | Induces VEGF expression and has angiogenic and neurotrophic actions | II                  |
| MCC-257§        | Sialic acid derivative           | Enhances endogenous neurotrophic support               | II                  |

*Drug for which the indication is angiopathy. †Drug for which the indication is nephropathy. §Drug for which the indication is retinopathy. ‡Drug for which the indication is neuropathy. Information was obtained from the database maintained at the ClinicalTrials.gov website. AGE, advanced glycation end products; PARP, poly(ADP-ribose) polymerase; PPAR, peroxisome proliferator-activated receptor; RAGE, receptor of AGE; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

Streptozotocin (STZ). A glucosamine-nitrosourea compound, originally isolated from Streptomyces achromogenes, that enters the pancreatic β-cell by a glucose transporter system and damages DNA by alkylation, leading to cell death and subsequent insulin deficiency. STZ is used to induce type 1 diabetes in animals and provides the most widely studied models of diabetic complications.

Renal glomerulus. Capillary bed of the renal corpuscle from which plasma is extruded and filtered before entering the tubular system of the kidney. The filtering system includes the endothelial cells of the capillary, the glomerular basement membrane, podocytes and associated mesangial cells. Disruption of one or more types of these cells can impair blood filtration and contribute to diabetic nephropathy.

nephropathy. These include early development of renal hypertrophy, progressive increases in albuminuria and changes to the renal ultrastructure, such as mesangial expansion and glomerular basement membrane thickening. Unfortunately, more advanced renal disease, specifically overt proteinuria, renal impairment and advanced structural lesions, are not prominent in this model. New models, such as the Akita and OVE26 mice, have been developed to address this concern and show more advanced lesions. Whether these new mouse strains will ultimately prove to be more useful preclinical models for testing new treatments remains to be determined, and much emphasis is currently being placed on the genetic background of the mice. For example, it has been suggested that manifestations of nephropathy in the Akita mouse model can differ depending on the genetic background of the mice.

Mechanisms and interventions derived from animal models. Much of the initial research into diabetic nephropathy relied on detailed characterization of the progression of the disease at the functional and ultrastructural levels. These studies identified the phase of microalbuminuria or incipient diabetic nephropathy, characterized by modest increases in urinary excretion of albumin. It was then shown that this phase predicts the subsequent development of overt renal disease and is associated with key structural changes to the renal glomerulus.

Recent studies have explored potential molecular and biochemical mechanisms that could be responsible for the progression of renal lesions in diabetes. Initial research focused on the mesangial cells within the glomerulus, but changes in other glomerular cells, including podocytes, and progressive injury to the tubulointerstitium have subsequently been described.
Glucose-dependent pathways (FIG. 2), such as advanced glycation⁷⁹, are clearly important components in the pathogenesis of diabetic renal disease and accompany organ-specific mechanisms involving disruption of vasoactive hormone pathways, such as the RAS⁸⁰. Glucose and AGE-mediated activation of RAGE⁹⁹,¹⁰⁰, key intracellular signalling molecules such as PKC and mitogen-activated protein kinase, and angiotensin II also have a central role in this disease⁹⁹. Furthermore, certain pro-sclerotic growth factors, such as transforming growth factor-β (TGFβ) and connective tissue growth factor (CTGF), seem to promote extracellular matrix accumulation, a cardinal structural feature of the kidney in patients with diabetes. It is also becoming increasingly apparent that metabolic and haemodynamic pathways not only interact through common mediators, such as intracellular signalling molecules and growth factors, but can directly interact with each other. For example, angiotensin II can enhance AGE accumulation in the kidney and AGE can directly modulate expression of key components of the RAS⁹⁹. Thus, it seems that metabolic and haemodynamic stimuli, triggered by the diabetic milieu, interact to amplify injury and perpetuate the progression of renal damage in diabetes (FIG. 3).

**Clinical development of therapeutics.** Over the past decade, there have been important advances in understanding the pathogenesis of diabetic nephropathy that have prompted ongoing clinical trials of new agents that are designed to delay and reverse diabetic renal disease. Efficacy of agents that influence advanced glycation pathways (such as soluble RAGE)⁸⁰, inhibitors of renal AGE accumulation (such as the putative cross-link-breaker alagebrium)⁸¹, inhibitors of PKC activation (such as ruboxistaurin, an inhibitor of PKCβ)⁸² or inhibitors of vasoactive hormone pathways (such as endothelin antagonists) in animal models have all stimulated new clinical trials. Some of these clinical trials have now been completed⁸³, whereas others are still in progress⁸⁴ [TABLE 1]. These trials are focusing primarily on albuminuria as an index of nephropathy, as well as assessing the decline in renal function. Because diabetic nephropathy takes many years to evolve and only occurs in a minority of subjects, these clinical trials have often been difficult to complete. Moreover, the US Food and Drug Administration currently considers renal impairment, and not proteinuria, as the appropriate renal end point of diabetic nephropathy. This requires studies that often involve more than 1,000 patients and a follow-up of up to 5 years⁸⁵,⁸⁶. Such studies are difficult to carry out in terms of recruitment and are expensive.

It is hoped that surrogate markers such as albuminuria may be accepted for approval by regulatory agencies to register drugs as renoprotective in diabetes⁸⁷, although the finding that some subjects with diabetes develop renal impairment in the absence of albuminuria⁸⁸ suggests that serial assessment of urinary albumin excretion may also not be ideal. Many research groups are trying to identify alternative biomarkers of renoprotection, and much of this work focuses on urine proteomics⁸⁹. This research is at an early phase, and a biomarker has not yet been established. Ultimately, the goal is to develop more robust strategies for monitoring diabetic nephropathy and in particular to generate a more reliable approach to identifying truly renoprotective regimens that not only decrease albuminuria but also reduce renal morphological injury and preserve renal function.

**Diabetic retinopathy**

Diabetes is the leading cause of new cases of blindness among adults aged 20–74 years⁸⁰. It is characterized by a range of retinal lesions and abnormalities that indicate vascular damage (capillary microaneurysms, capillary degeneration, increased vascular permeability and new vessel formation) and death or dysfunction of the neural retina (‘cotton wool spots’, alterations in retinal electrophysiology, loss of colour or hue discrimination). Clinically,
Microaneurysm
Small, focal points of damage to a blood vessel (usually capillary) wall, leading to pressure-induced swelling that may cause the vessel to rupture and allow leakage of blood (haemorrhage) into the surrounding environment.

Neovascularization
Growth of new blood vessels. Can be beneficial when blood vessels have been damaged, but can cause diabetic complications such as proliferative retinopathy if uncontrolled or inappropriate.

Oedema
Swelling of the extracellular tissue space by influx of fluid in response to the accumulation of osmotically active molecules. Oedema has a protective role following tissue injury but can also cause damage if it is prolonged or develops inappropriately.

Giall cell
A non-neuronal cell of the nervous system, including Schwann cells (in peripheral nerve), astrocytes and oligodendrocytes (in brain and spinal cord) that regulates the environment surrounding neurons. Smaller microglial cells are scavengers of cellular debris that can accumulate in the nervous system, particularly following damage.

Animal models of retinopathy. Numerous species, including monkeys, dogs, cats, pigs, hamsters, rats and mice, have been used as models for the study of diabetic retinopathy. All mammalian species studied to date develop at least the early stages of retinopathy, including degeneration of retinal capillaries. Development of retinopathy in these models occurs more rapidly in monkeys, dogs, cats, pigs, hamsters, rats and mice compared to humans, with early lesions developing within months to years of the onset of diabetes. The severity of retinal disease increases with duration of diabetes, but remains mild compared with that seen in many patients with diabetes. This is due in part to the limited lifespan of laboratory animals. With the exception of occasional contrary claims, diabetes alone has not been found to cause pre-retinal (intravital) neovascularization in any animal model. Again, this is probably due in part to insufficient severity of capillary degeneration and other lesions during the limited time over which these models have been studied. No data have yet been generated to suggest that the current animal models are inappropriate reflections of human diabetic retinopathy because they are mechanistically inadequate. Instead, the development and appearance of early retinopathy is similar in many diverse animal models of diabetes, in which the differences in the severity of retinopathy are related to the differences in the severity of hyperglycaemia.

Investigators who are interested in studying or inhibiting retinal neovascularization in vivo have turned to non-diabetic models in which retinal neovascularization occurs after branch vein occlusion, oxygen-induced retinopathy or overexpression of growth factors such as vascular endothelial growth factor (VEGF) or insulin-like growth factor in the eye. Because degeneration or occlusion of retinal capillaries in diabetes has been closely linked to eventual retinal neovascularization in humans, inhibition of diabetes-induced capillary degeneration is also used to evaluate therapeutic interventions.

Retinal neurons, particularly the retinal ganglion cells that connect the eye to the brain, have also been reported to degenerate in diabetic rats and some strains of diabetic mice. Diabetes-induced retinal neuron loss seems to begin before death of retinal vascular cells occurs but is not dramatic in these models, again perhaps owing to the short duration of the study. Despite this progressive neuronal loss, animal models have not yet been found to show evidence of visual impairment or blindness because of diabetes.

There remain important gaps in our understanding of the pathogenesis of retinal neovascularization in diabetes, the role of neural dysfunction and neurodegeneration in loss of vision and in the translation of efforts to inhibit retinal capillary degeneration in animal studies into the prevention of vision loss in patients with diabetes. Many cell types, both within and outside the retina, are likely to contribute to the development of diabetic retinopathy, and it is becoming apparent that the interrelationship between the vascular, neural, myeloid and giall cells needs further study in both health and disease. Ongoing efforts to develop genetically modified animals may offer new ways to investigate the pathogenesis of diabetic retinopathy and identify new therapeutic targets.

Mechanisms and interventions derived from animal models. Although it is widely accepted that diabetic retinopathy is caused by poor glycaemic control, the progression of retinopathy does not reverse, or even immediately halt, when better glycaemic control is achieved. The observation that diabetic-like retinopathy develops following a long-term increase in galactose levels in the blood, even in the absence of diabetes, provides strong evidence that hyperglycaemia per se, as opposed to insulin deficiency or alterations in lipid profile that are characteristic of poor diabetic control, is sufficient to initiate the development of aspects of retinopathy in mice and rats. However, disorders that commonly parallel hyperglycaemia in patients with diabetes, such as altered blood pressure and dyslipidaemia, have been found to influence the rate of progression of retinopathy. In recent years, there has been a great increase in the number of potential therapies that are reported to inhibit the diabetes-induced degeneration of retinal capillaries and neurons as well as the increase in retinal vascular permeability in animals. These range from pan-complication therapies, such as aldose reductase inhibitors, to tissue-specific approaches, such as anti-VEGF agents. Likewise, the range of therapies that have been found to inhibit retinal neovascularization in non-diabetes animal models is expanding and includes both those with potential efficacy against many diabetic complications, such as PKCβ inhibitors, and those that specifically inhibit mechanisms of retinopathy, such as blocking VEGF signalling.

A large group of diabetes-induced biochemical and physiological abnormalities in the retina, which were previously regarded as being independent of each other, are now known to associate in the context
of inflammation\textsuperscript{103,113}. The non-proliferative stages of diabetic retinopathy include altered vascular permeability and function, vascular degeneration, and neural dysfunction and degeneration. The interplay between these abnormalities is an ongoing area of research, and it is conceivable that individual therapies might not have comparable effects on each of these different areas.

**Clinical development of therapeutics.** Numerous clinical studies over the past several decades have provided strong evidence that surrogate markers that quantify the severity of retinopathy, such as the Early Treatment of Diabetic Retinopathy Study (ETDRS) retinopathy grading scale\textsuperscript{114,115}, can predict progression of the retinopathy and can indicate inhibition of disease progression. Randomized, multi-centre clinical trials have similarly provided valuable data on the ability of treatments, such as those providing improved glycaemic control, to inhibit the development and/or progression of retinopathy. Intensive treatment to normalize blood glucose levels resulted in a 63\% reduction in retinopathy progression and a significant inhibition of other microvascular complications\textsuperscript{26,136} in patients with diabetes. Furthermore, laser photocoagulation reduced the risk of severe visual loss by more than 50\% in the eyes of patients with high-risk characteristics\textsuperscript{17}.

On the basis of positive preclinical studies\textsuperscript{118}, a clinical study recently assessed the role of the angiotensin II antagonist candesartan in both type 1 and type 2 diabetes with or without early diabetic retinopathy\textsuperscript{116,120}. Overall, this agent had a positive effect, with reduced progression and in some groups regression of retinopathy as measured by the ETDRS scale. There is also increasing evidence of benefits from intravitreal injections of steroids such as triamcinolone. Often in conjunction with laser photocoagulation on vision-threatening retinopathy, including diffuse macular oedema\textsuperscript{121,122}. However, there are risks associated with intraocular steroid therapy, including increased intraocular pressure and enhanced cataract formation. Several other clinical trials have not produced the outcomes that were hoped for, probably owing in part to insufficient study duration, slow development or progression of retinopathy, insufficient inhibition of the therapeutic target or a lack of involvement of the therapeutic target in the pathogenesis of the retinopathy\textsuperscript{123-126}.

The development of non-invasive methods to assess the vascular and neural components of the eye is offering new opportunities to investigate the pathogenesis of retinopathy and to document responses to therapeutic intervention. Directed therapies against abnormalities that are believed to play key parts in the later stages of diabetic retinopathy, including inhibitors of PKC\textsuperscript{127,128} or VEGF\textsuperscript{129}, are providing therapeutic tools to inhibit clinically important aspects of retinopathy in patients with diabetes (TABLE 1). However, it is important to recognize that there is a disconnection between what is studied in animal models using high resolution microscopy and isolated tissue preparations, and what can currently be measured in patients with diabetes using colour fundus photographs, fluorescein angiograms and retinal function tests. New techniques, such as optical coherence tomography\textsuperscript{130} and magnetic resonance imaging\textsuperscript{131,132}, may be useful for both animal and clinical investigations and will hopefully lead to more interaction between preclinical and clinical scientists.

**Diabetic neuropathy**

Diabetic neuropathy affects the somatic and autonomic divisions of the peripheral nervous system, and there is a growing appreciation that the spinal cord\textsuperscript{133} and higher central nervous system (CNS)\textsuperscript{134} may also be damaged. Over half of all patients with diabetes develop some form of neuropathy\textsuperscript{135}, resulting in sensory loss, pain and autonomic dysfunction. These manifestations of neuropathy, along with their contribution to impaired wound healing and cardiovascular and erectile dysfunction, can severely reduce a patient’s quality of life. As with the other complications discussed above, duration of diabetes and lack of adequate glycaemic control are important risk factors for neuropathy in both type 1 and type 2 diabetes\textsuperscript{26,136}.

No therapy for diabetic degenerative neuropathy, other than maintenance of normoglycaemia, is approved by regulatory bodies in Europe and the United States, and current pain management strategies are not consistently effective and do not target the causes of diabetes-induced pain. The development of new therapies has been hindered by an incomplete understanding of the aetiological mechanisms involved, which is largely a reflection of the lack of a suitable animal model.

**Animal models of neuropathy.** Most studies have been carried out in rodent models of type 1 or type 2 diabetes, in which hyperglycaemia is induced by genetic, pharmacological or dietary manipulations. Short-term rodent models of diabetes quickly develop a slowing of nerve conduction but generally lack overt demyelination and fibre loss in nerve trunks, which are prominent features of clinical diabetic neuropathy\textsuperscript{136}. Longer durations of diabetes may produce discernable pathology in nerve trunks of some rodent models\textsuperscript{137,138}, and the retraction of small sensory fibre terminals in the skin of short-term rodent models of diabetes\textsuperscript{139,140} offers a measurement of nerve pathology that can be used in drug screening assays. The current rodent models of diabetic neuropathy may best reflect initial biochemical and functional disorders that precede degenerative neuropathy and could be useful for understanding early pathogenic events. However, there remains a prominent knowledge gap regarding the mechanisms of demyelination and neurodegeneration, such that a leap of faith has been required when translating any therapy that targets degenerative neuropathy from preclinical studies to clinical trials.

The lack of overt degenerative neuropathy in most rodent models of diabetes may be due to their short lifespan and physically shorter axons. Larger, long-lived diabetic animals have therefore also been used as alternative models. Diabetic dogs develop a slowing of nerve conduction and corneal hyposensitivity after years of hyperglycaemia\textsuperscript{41,142}, but degenerative neuropathy is minimal\textsuperscript{143}. Diabetic monkeys were recently shown to have epidermal nerve fibre loss\textsuperscript{144}, and studies of diabetic domestic cats have identified a degenerative neuropathy...
production of neurotrophic factors have also shown some success in diabetic rodents\textsuperscript{154–156}. Most recently, gene therapy to facilitate endogenous production of nerve growth factor\textsuperscript{157,158}, hepatocyte growth factor\textsuperscript{159}, neurotrophin 3 (REF 160) or VEGF\textsuperscript{161,162} has been explored. Advancement of this approach must clearly incorporate the goal of delivering localized and regulated production of such factors.

Impaired signalling through insulin receptors, whether driven by insulin deficiency or resistance, is also emerging as a possible primary pathogenic mechanism that contributes to diabetes-induced damage to the nervous system. Peripheral nerves have insulin receptors\textsuperscript{163}, and insulin treatment regimes that do not modulate circulating glucose levels can protect against neurochemical\textsuperscript{164}, behavioural\textsuperscript{165} and functional\textsuperscript{166,167} indices of neuropathy in diabetic rodents. This suggests that insulin acts as a support factor for peripheral nerves.

As well as impeding neurotrophic support, reduced insulin signalling can also activate neurotoxic mechanisms. This is becoming increasingly apparent in the CNS, where deficient insulin signalling has been linked to accumulation of amyloid-$\beta$ and hyperphosphorylation of the neuronal protein tau in models of Alzheimer’s disease (REF 168). Amyloid-$\beta$ and hyperphosphorylated tau also accumulate in the brain of diabetic rats\textsuperscript{169} and mice\textsuperscript{170,171}, in which there is evidence of synaptic and neuronal loss. There is continuing debate regarding the extent to which patients with type 1 and type 2 diabetes are prone to developing cognitive impairments and manifestations of dementia\textsuperscript{172,173}. Nevertheless, the apparent convergence of pathogenic mechanisms for these two common diseases of ageing on defective insulin signalling is attracting considerable interest\textsuperscript{174} and may reveal new targets for therapeutic intervention.

Therapeutic strategies to treat the pain associated with diabetes have traditionally been drawn on experience from other neuropathic pain states rather than targeting diabetes-specific mechanisms; an example of this is the widespread use of tricyclic antidepressants\textsuperscript{175}. The anticonvulsants gabapentin and pregabalin\textsuperscript{176,177} and the antidepressant duloxetine\textsuperscript{178} have been used for alleviating diabetes-induced neuropathic pain, albeit with caveats concerning their efficacy, side effects, cost effectiveness\textsuperscript{179} and a lack of effect on the underlying degenerative neuropathy. Although these treatments have emerged from broad anti-neuropathic pain programmes, preclinical studies suggest that a wider therapeutic window may exist in diabetes because of the increased expression of the presumed targets\textsuperscript{180,181}. Reports that diabetes increases the expression of other potential targets, such as peripheral $\kappa$-opioid receptors\textsuperscript{182} and spinal cyclooxygenase 2 (REF 183), may provide additional avenues for drug development.

**Clinical development of therapeutics.** Many agents have entered clinical trials to treat diabetic neuropathy. Twenty five years of failure to translate the mechanistic and therapeutic findings in animal models to a clinically effective treatment for diabetic degenerative neuropathy has prompted sharp divides in the research community.
Specifically, some researchers see the animal models as being inadequate tools for discovering effective therapeutics and others think that the clinical trials have been poorly designed and executed or have relied on inappropriate or unreliable end points; both views probably have some merit.

Approaches currently under investigation range from refinement of aldose reductase inhibition and other interventions that may have pan-organ efficacy to the neuron-specific targeting of neurotrophic support mechanisms (TABLE 1). Previous clinical trials have largely relied on the slowing of nerve conduction and on sensory testing as quantifiable predictors of progressive degenerative neuropathy. Sural nerve biopsies have also been used as a direct measure of nerve pathology, but the technique is invasive, difficult to quantify and does not allow multiple measurements. The continued failure to show an acceptable clinical efficacy of therapies that were developed from preclinical screening against slowing of nerve conduction in diabetic rodents could be due to mechanistic differences between species or the lack of reliable measures of early neuropathy.

One advance that may assist drug development studies is the emerging use of skin biopsies as a minimally invasive measure of small fibre distal degenerative neuropathy. Epidermal fibre loss can be detected even before the onset of clinically overt diabetes and is associated with functional indices of sensory loss. Although the technique has currently been restricted to the evaluation of small sensory fibres and not of myelinated sensory, motor or autonomic fibres that are also affected by diabetes, it offers the advantage of providing a direct measurement of fibre loss that may ultimately result in loss of thermal sensation. Repeated biopsies can be carried out and allow monitoring of the progression of neuropathy and the effects of drug treatment.

The use of corneal confocal microscopy to view changes in sensory nerve fibres in the eyes of patients with diabetes is also currently being evaluated against other measures of early neuropathy. Corneal confocal microscopy offers the additional advantage of being entirely non-invasive, which allows many sequential measurements. It also facilitates local and topical drug delivery and can be used to detect nerve regeneration. Skin biopsy and corneal microscopy may therefore prove useful additions to future clinical trials of drugs that target diabetic neuropathy.

Current and future challenges
It is likely that refinement of pancreatic transplantation and other approaches to maintaining long-term regulation of insulin and glucose levels, such as the artificial pancreas, will ultimately reduce the incidence and severity of diabetic complications. However, initial periods of poorly controlled diabetes can have a protracted negative effect on the subsequent protection that is afforded by improved glycaemic control, and numerous patients have, or are in the process of developing, diabetic complications. These caveats provide sufficient concern to support efforts directed at understanding the pathogenic mechanisms that cause diabetic complications and at developing therapeutic interventions.

The effects of diabetes on the organ systems described above emphasize that the main complications share numerous glucose-driven pathologic mechanisms. Targeting glucose-mediated vascular dysfunction with approaches such as inhibition of RAGE signalling, aldose reductase activity and oxidative stress is a particularly appealing therapeutic approach as it offers potential efficacy against multiple complications. Moreover, some of these targets may also have additional organ-specific effects that are independent of their impact on vascular function. For example, aldose reductase also localizes in Schwann cells of peripheral nerves and in cells of the kidneys and eyes, whereas RAGE expression is induced or increased by diabetes in each of these organs independently of its expression in blood vessels. Some treatments therefore have the potential to disrupt both universal and organ-specific pathogenic mechanisms.

The limitations of current rodent models of diabetic complications, which tend to show early metabolic and functional disorders but lack marked structural pathology, are a cause for concern. The use of these models is driven in part by the desire of researchers to accelerate the onset of complications in inexpensive species that are amenable to genetic manipulation. Although current rodent models are useful for identifying the initial pathogenesis of diabetic complications and for studying the effect of potential prophylactic and early interventional therapies, there is persistent uncertainty as to whether investigators are studying mechanisms and drugs that are pertinent to overt pathological damage in humans. Another recurring theme is the difficulty in designing viable clinical trials and identifying which end points should be used as indicators of therapeutic efficacy. It is clear that the regulatory bodies are challenged by conflicting requirements to establish consistent end points which can be used in trials that may take many years to complete, while needing to have the flexibility to adopt new surrogate biomarkers as they are identified and validated by preclinical and clinical studies.

The fact that diabetes disrupts such a diverse range of highly specialized organs also presents substantial challenges for drug development. The example of VEGF, for which inhibitors are being developed to treat diabetic retinopathy and increased expression provoked to treat neuropathy, illustrates the complexities that have to be considered when addressing individual complications within the context of a disease that impairs so many systems. Nevertheless, new animal models, creative drug delivery systems and improvements in clinical trial design and biomarkers will hopefully combine to accelerate the development of therapies for diabetic complications and improve approaches that are already being investigated. A single drug that is effective against all diabetic complications may not be a realistic goal. Therapeutic strategies that incorporate interventions targeting the glucose-mediated pathogenic axis outlined in FIG. 2, in combination with those that address organ-specific and glucose-independent mechanisms, may provide the most successful approach to drug development in this area.
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DATABASES

UniprotKB: http://www.uniprot.org

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FURTHER INFORMATION

6-Month safety and efficacy study of P488 in patients with type 2 diabetes and persistent albuminuria: http://www.clinicaltrials.gov/ct2/show/NC100287183?term=NC100287183&rank=1

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