Diabetic retinopathy (DR) is the main cause of blindness for working age populations in developed countries. It is estimated that the worldwide prevalence will exceed 200 million individuals by 2012. Intensive control of glycemia and blood pressure can reduce the prevalence of DR. Laser thermal destruction of the retina can prevent the progression of the disease and loss of vision. However, in addition to its limited efficiency this technique is associated to a high percentage of complications. Therefore, it is crucial to research new therapies utilizing novel action mechanisms. A greater understanding of the biochemical mechanisms involved in the onset and development of DR is essential for developing new therapeutic agents. This would be facilitated by the use of animal models, considering that the physiology of mice, rats and other animals (such as monkeys) can be quite similar to that of humans.

It is known that chronic glycemia in diabetes is the main cause of damage to blood vessels, neurons and glial cells in the retina. However, the complexity in creating a truly representative model of human DR exceeds the development of methods for raising sugar levels in the serum of animals. None of the currently available animal models comprises all the characteristics of DR. Each model reproduces a relevant aspect of the disease and also includes unique physiological characteristics that influence the interpretation of experimental results.

Molecules can have very different functions in laboratory animals and can be expressed in different cell types. These characteristics can be unique to an animal species with a specific genetic line. Another deficiency inherent in animal models is the specificity of each case. The majority of animals used in medical research are of endogamous origin. In contrast, human patients are genetically heterogeneous. But the origin of their disease, together with their responses to treatment, can vary enormously. A specific line of mice or rats can be considered to represent in the majority of cases a single sub-group of human patients. The validation of therapies in more than one animal model should resolve many of the problems specific to a species and a genetic line. A drug which has an effect in transgenic rats and mice is more likely to modify a universal mechanism of the disease which is also more likely to be present in humans.

The majority of DR models imitate diabetes type 1, caused by the destruction of the beta cells of the pancreatic islets. Rats and mice injected with streptozotocin (STZ) or alloxan lose their beta cells and become insulin-dependent. This approach reproduces some of the early symptoms of DR and has the advantage that the onset of diabetes can be defined as the moment in which the toxin is injected. Many variations have been identified between species and also within the same species, both in the retinal biochemistry as in the histopathological responses to identical diabetic «insults». Rats injected with STZ lose the retinal pericytes and capillaries, develop thickening of the vascular basal membrane and an increased vascular permeability. In rats injected with STZ, physiological and biochemical changes in the retina appear between one and two months after hyperglycemia. Müller cells exhibit changes after 2-5 months of experimental diabetes. Capillary obliteration first becomes obvious after six months of diabetes. In turn, nonvascular abnormalities (neuronal and glial) induced by diabetes precede the development of changes in vascular cells in rats and may contribute to the pathogenicity of the vascular disease in this model (1).
The power of the mice models in DR lies in the ease with which genetic changes can be introduced to explain the molecular mechanisms involved in the onset and development of the disease. However, STZ-induced diabetes in mice is less successful than in rats due to the resistance of mice to this toxin. Mice injected with STZ develop vascular alterations which are characteristic of early DR, specifically the induction of acellular capillaries, apoptosis of vascular cells and the formation of «ghost» pericytes six months after the induction of diabetes, regardless of the lack of significant neuronal loss and persistent glial activation (2). This suggests that the processes which give rise to vascular changes are in some way different to those which initiate non-vascular changes in mice, thus allowing us to study vascular changes in an isolated manner.

There also models which develop type 1 diabetes spontaneously, such as the Ins2Akita mouse model (3), which has a dominant mutation in the insulin 2 gene. This causes a faulty withdrawal of insulin, the death of beta-pancreatic cells, systemic hypoinsulinemia and hyperglycemia at four weeks of life. The retina of heterozygotic males exhibit vascular, neural and glial abnormalities similar to the changes detected in rats injected with STZ. Even so, the use of the Ins2Akita mouse has several advantages: Firstly, the dominant mutation allows us to study heterozygotic animals; secondly, the mice are fertile and reproduce better than rats; thirdly, their diabetes comprises a deficiency of stable insulin and thus can be maintained in a less catabolic condition without exogenous insulin; and fourth, the diabetes onset of mechanism does not involve immunological alterations.

The «true» pre-retinal neovascularization has only been demonstrated in dogs fed with high amounts of galactose (4), a model with high levels of sugar in plasma and a prolonged life, allowing the development of vascular changes in the retina both at the early and late stages of retinopathy. There are relatively few studies comparing the development of retinopathy in diabetes type 1 and 2, however, it is likely that the differences between them are very small. There are only a few models of true type 2 diabetes, which is associated to obesity and insulin resistance. The Zucker obese rats, the Tori diabetic fat rat and the Otsuka Long-Evans Tokushima fat are examples of rats which develop type 2 diabetes spontaneously. Ob/ob 9 and KKAY rat are good models of some metabolic changes in diabetes type 2. However, neither rats nor mice seem to develop retinal changes similar to diabetic retinopathy in humans with diabetes type 2.

The only currently available animal model which exhibits expressions of diabetes similar to humans is a monkey with a prolonged type 2 diabetes which develops loss of capillaries, intra-retinal hemorrhage and microaneurysms (5). Even considering the high cost in resources and time, large animal species should be considered due to their possible role as «functional models». They represent a unique physiological and natural preconditioning as well as highly valuable experimental advantages for the development of alternative models for diabetic retinopathy study.

Even though most of the research on diseases is carried out in vivo, Huub Kreuwel de Entelos and Mark Atkinson from Florida University have developed a virtual model joining the major organs of a virtual mouse model. This mouse consists of interconnected modules representing cells, tissues and organs. Mathematical algorithms for each tissue make up sets of parameters which were determined in previous mouse studies. The potential advantages of this model include the possibility of following continuous levels of regulator cells, insulin and other biomolecules, establishing the best doses for drugs as well as reconciling apparently contradictory result of studies. Modifications in the program would allow for the representation of additional mouse lines and in the future it could be used for research in DR.

As currently there isn’t a single model that fully reproduces the mechanisms involved in human DR, each researcher has to choose the models that reproduce a relevant aspect of the disease.

Considering the formidable obstacles for developing models portraying the human situation, it is not surprising that the correlation between studies with animal models and human patients has been less than perfect. Treatment with kinase-C protein (PKC) inhibitors is a relevant example. It was demonstrated that it protected in a relative or complete manner against macular edema in animal DR models. However, in the past 10 years it appeared that PKC inhibitors have little protective effect on humans or none at all. The absence of agreement in the effect of these agents on animals and humans could be due to artifacts of animal models, differences in the physiology and the therapeutic effects between lab animals and humans, and/or discrepancies between the «equivalent status of the disease»
between animals and humans. The onset of DR in humans is defined with the development of clinically identified vascular changes. In animals, the «retinopathy» induced by diabetes is considered to begin much earlier, when the lab parameters begin to change. It is known that the timeliness of therapy initiation is critical to revert the changes induced by diabetes in lab animals. This suggests that some therapies may have failed in humans because the treatment was initiated at the «wrong» time.

Until now, the path from animal tests to the development of DR therapies in humans has faced considerable difficulties. However, the use of animals will continue to make a substantial contribution to the study of molecular mechanisms of DR pathophysiology. The consideration of the differences between animals and humans should facilitate the discovery of clinically beneficial treatments (hopefully, in the near future!).

REFERENCES