TGF-β and CTGF: pro-fibrotic factors in diabetic retinopathy

van der Geest, R.J.

Citation for published version (APA):
Introduction and Aim of the Thesis
Introduction and aim of the thesis

The worldwide prevalence of diabetes is increasing, with an estimated 285 million affected people in 2010. This number is expected to keep growing, with the increasing frequency of obesity and life span. Diabetic retinopathy (DR) is a common microvascular complication of diabetes, with about a third of people with diabetes having signs of DR, and a third of the latter population having vision-threatening retinopathy, i.e. severe retinopathy or macular edema. It is a leading cause of vision loss not only in the working-age population, but also in the elderly.

An example of normal retina is shown in Figure 1. The clinical features of DR, as detected by ophthalmoscopy, begin with microaneurysms and progress to exudative changes such as haemorrhages and hard exudates due to leakage of lipoproteins (Figure 2), ischaemic changes seen as cotton-wool spots that represent infarcts of the retinal nerve-fiber layer, and development of intraretinal microvascular abnormalities and venous beading. In this non-proliferative form of DR (NPDR), vascular leakage and macular ischaemia can cause vision loss.

With increasing vascular damage and subsequent ischaemia of the retina, new vessels may develop on the optic disc and the retina, called neovascularization (Figure 3), which hallmarks the progression to proliferative diabetic retinopathy (PDR). These newly formed vessels easily rupture, causing vitreous haemorrhage, or may progress to form fibrovascular tissue and finally fibrotic scarring, a process that we proposed to call the angio-fibrotic switch. In this phase traction on the retina will develop, resulting in retinal detachment and blindness.

The vision-threatening clinical manifestations of DR are preceded by an asymptomatic pre-clinical phase (PCDR). During the 5-15 years of PCDR, hyperglycaemia induces a number of pathological changes in the retina, among which diffusely increased permeability, thickening of the basal lamina (BL) of retinal capillaries, loss of pericytes, degeneration of endothelial cells and neurons, and the development of acellular capillaries. The acellular capillaries eventually develop into expanding areas of capillary non-perfusion, retinal ischaemia and other clinical signs of DR.

The exact sequence of the pre-clinical events and their relative importance in the development of DR are not clear yet. Loss of pericytes is considered to be an important early event, as pericytes induce maturation of capillaries, maintain capillary stability and regulate homeostasis of the endothelium. Loss of pericytes is followed by reduced numbers of endothelial cells and ultimately to the formation of non-perfused acellular capillaries. Thickening of the BL around capillaries of the inner retina is another early structural change in PCDR. It is the result of extracellular matrix (ECM) remodeling leading to increased deposition of BL components such as collagen type IV, laminin and fibronectin (FN), and occurs in both diabetic humans and animals. Inhibition of diabetes-induced BL thickening in rodent models by modulation of BL components has been shown to prevent other early changes, suggesting that BL thickening may be critical in the further development of DR into the clinical phase.

Several biochemical and other mechanisms associated with hyperglycaemia have been proposed to be central in the pathogenesis of DR, leading to several often conflicting concepts. These include accumulation of advanced glycation end products (AGEs), increased oxidative stress, protein kinase C activation, increased adherence of leukocytes, and upregulation of the expression of growth factors such as insulin-like growth factor and vascular endothelial growth factor (VEGF).
There is extensive evidence that VEGF is a major growth factor causing vascular leakage and neovascularization in conditions with retinal ischaemia, such as DR. However, VEGF may also play a role in earlier phases of DR, as it is overexpressed in experimental PCDR, and is suggested to contribute to BL thickening, possibly in concert with growth factors that have pro-fibrotic properties, such as transforming growth factor-beta (TGF-β) and connective tissue growth factor (CTGF).

TGF-β, of which three isoforms have been identified in mammals (i.e. TGF-β1, 2 and 3), belongs to the TGF-β superfamily of growth factors, which comprises more than 30 members, including bone morphogenetic proteins (BMPs) and activin. TGF-β has multiple functions which, in addition to its role as a major inducer of ECM production, include downregulation of cell proliferation and upregulation of cell differentiation, immune cell modulation and cytokine production. TGF-β can induce expression of CTGF, platelet-derived growth factor, fibroblast growth factors, and VEGF. A causal role of TGF-β in capillary BL thickening and diabetic microvascular disease has been demonstrated in organs other than the eye. TGF-β signaling has also been implied in the pathogenesis of DR. For instance, it was shown that drugs that are effective in the suppression of experimental DR had in common that upregulation of genes of the TGF-β pathway was suppressed.

CTGF is a member of the CCN family of matricellular proteins and is also known as CCN2. It is a potent pro-fibrotic factor involved in ECM synthesis, and its levels are increased under diabetic conditions. CTGF functions as a downstream mediator of TGF-β signaling and may act as a co-factor for the pro-fibrotic activity of TGF-β, but can also induce ECM synthesis independently.

Previous work from our group points at an important role of CTGF in the pathogenesis of both PCDR and PDR. In the retina of rodents, CTGF is upregulated in streptozotocin (STZ)-induced diabetes, as well as after systemic infusion with AGEs. In PCDR, mice lacking one functional CTGF allele (CTGF mice) did not show the BL thickening that was observed in diabetic wild type mice with experimentally-induced diabetes. CTGF protein expression is relatively higher in pericytes in the retina of diabetic humans with early DR compared to normal retina. In patients with proliferative DR (PDR), CTGF is associated with fibrosis and, in a critical balance with VEGF, with the induction of the angio-fibrotic switch. Although CTGF can be induced in cultured cells by other factors than TGF-β, e.g. high glucose levels and AGEs, our findings could also be explained by involvement of TGF-β in diabetic retinal BL thickening.

The evidence supporting an important role of CTGF in DR is reviewed in chapter 2.

The aim of this thesis is to expand our knowledge on the role of TGF-β, CTGF and VEGF and their interactions in different phases of DR.

We hypothesized that TGF-β, CTGF and VEGF all possess pro-fibrotic properties in PCDR and are involved in ECM remodelling of retinal vascular cells, and/or thickening of the BL surrounding the retinal capillaries. To investigate this we used cultures of retinal endothelial cells and pericytes to study the effects of VEGF-A (chapter 3) and TGF-β (chapter 4) on pro-fibrotic and ECM-related genes and proteins. We also studied the effect of VEGF on pro-fibrotic growth factors and ECM genes in rodent retina (chapter 3). Furthermore, we explored the role of CTGF not only in BL thickening, but also in other pre-clinical changes in the diabetic rodent retina (chapter 5).

To test our previously proposed model of the balance between CTGF and VEGF in the angio-fibrotic switch in patients with PDR, we studied these growth factors and their association with
fibrosis in vitreous of patients after intravitreal anti-VEGF treatment (chapter 6). Furthermore, to study the clinical course of PDR, we measured levels of TGF-β, CTGF and VEGF levels and of the ECM-related protein TIMP-1 in vitreous of diabetic patients without DR and patients with PDR, and associated their levels with clinical scores of retinal fibrosis and neovascularization (chapter 7).

In chapter 8, we discuss the findings of the studies described in this thesis in relation to the retinal pathology in PCDR and PDR.

REFERENCES


Figure 1. Fundus photograph (A) and fluorescence angiogram (B) of normal eye. Normal retinal vessels are present.

Figure 2. Fundus photograph (A) and fluorescence angiogram (B) of an eye of a patient with non-proliferative diabetic retinopathy. Note intra-retinal hard exudates (A, arrowheads) surrounding areas of leaking microaneurysms (B, white arrows).

Figure 3. Fundus photograph (A) and fluorescence angiogram (B) of an eye of a patient with proliferative diabetic retinopathy. Note preretinal neovascularisation (black arrow) on the optic disc (A), which is extensively leaking fluorescein (B). White arrows, microaneurysms.