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REVIEW

Pathogenesis of renal microvascular complications in diabetes mellitus

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INTRODUCTION

Microvascular disease is the main determinant in the development of late complications in diabetes mellitus. This is obvious for diabetic nephropathy[1,2] and retinopathy,[3,4] but changes in the microcirculation may also play an important role in the pathogenesis of diabetic neuropathy.[5,6] Although the pathogenesis of diabetic microangiopathy is incompletely understood, it is likely that it involves an interaction between metabolic and functional/haemodynamic factors. Together with genetic and environmental factors this results in the development of microvascular complications.

EPIDEMIOLOGY

Diabetic nephropathy is a major cause of morbidity and mortality in patients with type 1 as well as type 2 diabetes. Diabetic nephropathy is characterised by specific morphological changes including glomerular basement membrane thickening, mesangial expansion and glomerular and tubulo interstitial sclerosis. The clinical syndrome of diabetic nephropathy consists of proteinuria, hypertension and a progressive decrease in the glomerular filtration rate. Various studies reveal cumulative incidence rates for diabetic nephropathy of 20 to 40%,[7-9] but it is suggested that the incidence is declining.[10] Although the incidence of diabetic nephropathy among diabetic patients is decreasing, the prevalence of nephropathy among the population as a whole is dramatically increasing, predominantly due to improvement of hypertension and coronary heart disease, and due to an increase in prevalence of type 2 diabetes mellitus. The risk of diabetic nephropathy among type 2 diabetic patients with progression to end-stage renal disease is comparable with that in type 1 diabetes mellitus.[11,12] The peak incidence of diabetic nephropathy is between 10 and 17 years after the onset of diabetes.[8,13] Thereafter, the incidence of diabetic nephropathy declines rapidly. The first clinical manifestation of diabetic nephropathy is microalbuminuria, defined as a urinary albumin excretion rate of 20 to 200 μg/min. Microalbuminuria is associated with other microvascular complications as well as with cardiovascular disease suggesting some common pathophysiological mechanisms.[7,14]

PATHOPHYSIOLOGY

The pathophysiology of renal microvascular complications in diabetes mellitus consists of an intensive interplay of metabolic and functional/haemodynamic factors that underlies the structural changes of the microvasculature.

Structural changes

These structural abnormalities in diabetic glomerulopathy include an increase in basement membrane thickness, mesangial expansion with accumulation of extracellular matrix components (ECM) and glomerular fibrosis. There is an inverse correlation between heparan sulphate proteoglycan (HSPG) expression and mesangial expansion in diabetic glomerulopathy,[15] stressing the importance of dysregulation of ECM synthesis, which seems crucial for the development of renal microvascular complications. Furthermore, the extent of ECM accumulation correlates strongly with the degree of tubulointerstitial fibrosis and

of renal failure and proteinuria.[16-18] Tubulointerstitial injury is probably a major feature of disease progression.[19] Chronic interstitial injury usually follows the onset of glomerular proteinuria. Both structural lesions are probably linked via the increase in glomerular permeability and ultrafiltration of bioactive (growth) factors which can be held responsible for inducing and/or aggravating ECM production and renal fibrosis.

Metabolic pathways

Several factors such as high glucose, intracellular polyols, non-enzymatic glycation products, hexosamines and vasoactive hormones are held responsible for the changes in regulation of the biosynthesis of matrix components composition and accumulation.[20] They stimulate the synthesis and release of growth factors and cytokines from resident renal cells, inducing cell proliferation and hypertrophy, as well as the production of extracellular matrix proteins.[21] Of these the profibrotic cytokine, transforming growth factor-β (TGF-β), has emerged as a key factor in the development of structural abnormalities in diabetic nephropathy.[22] *In vitro* experiments showed that glomerular mesangial cells, epithelial cells and interstitial fibroblasts increased their TGF-β expression when exposed to high glucose.[23,24] Also, *in vivo*, the expression of renal TGF-β is increased in experimental as well as in human diabetes.[25,26] In animal models, neutralising TGF-β antibodies prevented the increase in extracellular matrix components and the increase in mRNA encoding for type IV collagen α1 and fibronectin. Furthermore, anti-TGF-β almost completely prevented the fall in creatinine clearance in diabetic *db/db* mice.[27]

Except for ECM accumulation, it is suggested that changes in the heparan sulphate chains of HSPG in glomerular basement membranes and ECM play a role in diabetic renal disease. A decrease in heparan sulphate, the anionic side chain of HSPG, induces proteinuria, stressing the importance of heparan sulphate for the permselectivity of the glomerular basement membrane.[28] It has been suggested that proteinuria itself plays a pathogenic role in diabetic nephropathy.[29] However, in contrast to the prevention of the decrease in glomerular filtration rate, neutralising TGF-β antibodies did not prevent proteinuria in diabetic mice.[27] This could implicate that the detrimental effects of proteinuria on renal function are mediated via TGB-β or that proteinuria is just a consequence of permselectivity changes.

Nevertheless, heparan sulphate also determines the local concentration, compartmentalisation, stability and activity of certain growth factors and proteases, thus controlling ECM expansion.[30] Heparan sulphate is probably directly involved in the inhibition of TGF-β overexpression[31] and regulates the expression of decorin, an extracellular matrix protein that inactivates TGF-β.[32]

Hyperglycaemia

The link between ECM accumulation via TGF-β and other profibrotic cytokines and diabetes is met by metabolic changes characteristic for the diabetes state per se. The metabolic hypothesis suggests that microvascular complications develop as a direct consequence of hyperglycaemia. Several small, prospective, randomised intervention studies and the Diabetes Control and Complications Trial (DCCT)[33] have definitely proven that improved metabolic control achieving near-normoglycaemia can reduce the incidence of diabetic nephropathy. These studies revealed duration and severity of hyperglycaemia as major risk factors for the development of diabetic microvascular complications. Complete normalisation of blood glucose after pancreas transplantation even shows a regression of structural renal changes.[34]

The mechanisms by which hyperglycaemia gives rise to microvascular complications have slowly been unravelled in the last years, supported by a large amount of experimental as well as clinical data.

Advanced glycation end products

Advanced glycation end products (AGEs), which are formed by non-enzymatic glycation of proteins, accumulate in renal glomeruli.[35] Recent research has shown that AGE precursors (dicarbonyls, such as methylglyoxal) are formed intracellularly from intracellular hyperglycaemia. These precursors can react with amino groups of intracellular and extracellular proteins to form AGEs. AGEs are capable of inducing increased vascular permeability, enhancing protein and lipoprotein deposition, inactivating nitric oxide and promoting matrix protein synthesis and glomerular sclerosis.[36] This last mechanism is probably mediated by TGF-β.[37,38]

The clinical importance of AGEs in diabetic nephropathy is stressed by the role of AGE formation inhibitors such as aminoguanidine and ALT-946 and the so-called AGE cross-link 'breakers' (phenacylthiazolium bromide; ALT-711) for treatment or prevention of diabetic (renal) complications.[39,40]

Protein kinase C

An increase in intracellular glucose induces *de novo* synthesis of diacyl glycerol (DAG)[41] which activates protein kinase C (PKC). PKC is capable of phosphorylating a number of cellular proteins. Increased PKC activity modulates gene expression in mesangial cells, inducing extracellular matrix protein synthesis, especially of type IV collagen and fibronectin, which is mediated by TGF-β.[22,42] Furthermore, PKC activation is linked to mitogen-activated protein kinase (MAPK), which is important in the intracellular signal transduction processes leading to cell proliferation and hypertrophy.[43,44] Activation of PKC increases production of vasodilatory prostanoids leading to hyperfiltration.[45] Animal studies showed that blockade

of PKC by means of LY333531 reversed renal hyperfiltration and increased glomerular albumin permeability.[46] Treatment with a PKC inhibitor showed a reduction in urinary albumin excretion rates and prevented mesangial expansion observed in diabetic *db/db* mice, possibly through attenuation of glomerular expression of TGF-β.[47]

Polyol pathway

Hyperglycaemia induces an increased flux through the polyol pathway. Originally it was thought that intracellular formation and accumulation of sorbitol, mediated by aldose reductase, leads to increased intracellular osmolality and swelling of cells. However, just recently it has been shown that decreased levels of reduced glutathione (GSH), as a result of the reduction from glucose to sorbitol,[48] are responsible for the deleterious consequences by increasing intracellular oxidative stress (see below). Clinical trials suggested the potential usefulness of aldose-reductase inhibitors in preventing the progression of incipient diabetic nephropathy in patients with type 2 diabetes mellitus.[49]

Hexosamine pathway

In diabetes an increased flux of intracellular glucose through the hexosamine pathway results in increased N-acetylglucosamine (GlcNAc), by conversion from fructose-6-phosphate by the enzyme GFAT. Most probably, modification of transcription factors such as Sp1 by GlcNAc will lead to transcription of the gene for TGF-β.[49] Furthermore, GlcNAc modifies many other intracellular proteins such as eNOS activity[49] that may contribute to the pathogenesis of renal diabetic complications.

Reactive oxygen species as a common pathway

Recently, Nishikawa *et al.*[50] and Du *et al.*[51] demonstrated that the formation of AGEs, activation of PKC, and activation of the polyol pathway as well as the hexosamine pathway, are mediated by the production of reactive oxygen species (ROS). They showed that elevated (intracellular) glucose levels increase the production of ROS in mitochondria. This over-production of ROS was prevented by manganese superoxide dismutase or by an uncoupler of oxidative phosphorylation, by uncoupling protein-1, and completely prevented intracellular formation of AGEs, activation of protein kinase C, increase in polyol pathway flux and hexosamine pathway activation. They concluded that ROS production is a common pathway in the initiation of high glucose-mediated stimulation of the aforementioned pathways.
It is hypothesised that an excess of ROS inhibits GADPH (glyceraldehyde-3-phosphate dehydrogenase), a glycolytic key enzyme promoting shunting of upstream glucose metabolites into the aforementioned pathways.

Functional/haemodynamic pathway

Long-lasting poor metabolic control does not necessarily lead to diabetic microvascular disease. This means that the apparent protection of diabetic patients for nephropathy cannot solely be explained on the basis of better metabolic control. The haemodynamic hypothesis implies that due to haemodynamic alterations in blood flow and pressure, structural changes are provoked, which will result in the development of microvascular complications.

Flow/pressure (haemodynamic hypothesis)

Capillary hyperperfusion precedes the onset of diabetic renal microangiopathy.[2] This observation has led to the hypothesis that changes in systemic or local haemodynamics contribute to the development of diabetic nephropathy.[52]
Micropuncture studies revealed a range of haemodynamic alterations in diabetes: increased intraglomerular pressure, increased single nephron GFR and preferential afferent compared with efferent arteriolar vasodilation. These renal haemodynamic changes may be related to vasoactive hormones such as angiotensin II, endothelin, nitric oxide, locally active prostaglandins and kinins, and atrial natriuretic peptide. Furthermore, hyperglycaemia, glucagon, insulin, insulin-like growth factor and reduced sympathetic nerve activity may be involved in diabetic microvascular haemodynamic changes. These haemodynamic changes cause injury to the vascular wall, resulting in increased permeability, intima fibrosis, and vascular smooth muscle cell proliferation.[53]
Therapy aimed at reversing glomerular hyperfiltration, by controlling glucose concentration early in the course of the disease, dietary protein restriction, and antihypertensive therapy, may slow the rate of progression of the renal disease. Many pharmacological substances are currently being developed which block the effect of vasoconstrictory hormones or reduce the degradation of vasodilating hormones. Because most of the enzymes involved in production and degradation of these vasoactive hormones have considerable homology, substances are being developed which interact with more than one of these systems.

Renin-angiotensin system (RAS)

The therapeutic effects of angiotensin-converting enzyme (ACE) inhibitors and AT_1-receptor antagonists in decreasing the progression of microalbuminuria or macroalbuminuria stresses the importance of the renin-angiotensin system (RAS).[54-58] The decrease in progression of diabetic nephropathy by ACE inhibitors and AT_1-receptor antagonists was originally attributed to their ability to control systemic and intraglomerular hypertension. However, despite comparable reductions in systemic blood pressure, endothelin-receptor blockade was not as renoprotective as an ACE inhibitor, at least in rats.[59] Indeed, the aforementioned studies showed that effective blockade of angiotensin II action had favourable renoprotective effects that go beyond the blood pressure lowering effects of these drugs. Thus, it is likely

that some of these favourable effects might be related to the non-haemodynamic effects of angiotensin II. Angiotensin II induces smooth muscle cell growth, hypertrophy and proliferation of glomerular cells and stimulates the synthesis of ECM components, collagen and fibronectin. The angiotensin II-induced smooth muscle cell growth and the hypertrophic and fibrogenic responses are mediated by TGF-β.[60] Neutralising anti-TGF-β antibodies are able to prevent angiotensin II-stimulated production of ECM in *in vitro* studies with mesangial cells.[61] Furthermore, angiotensin II activates PKC in glomerular cells through AT_1-receptor stimulation.

Endothelial dysfunction

The normal endothelium has important regulating properties for vascular tone and is intimately involved in the regulation of vascular and renal permeability. It regulates the composition of ECM and the proliferation of smooth muscle and mesangial cells.[62] Therefore, endothelial dysfunction has been implicated in the pathogenesis of diabetic vascular disease. In non-complicated type 1 diabetic patients acetylcholine-induced endothelial-dependent vasodilatation is intact.[63] In contrast, diabetic microalbuminuria reflects widespread endothelial dysfunction. An increase in von Willebrand factor (vWF), a component of the endothelial cell membrane and a marker of endothelial damage, precedes the occurrence of microalbuminuria.[64] The mechanisms by which diabetes causes impaired endothelial function are largely unknown. In view of the haemodynamic changes in diabetes a biphasic process is proposed. As a vasodilator, nitric oxide (NO) is a candidate for mediating the increases in blood flow and capillary permeability that are observed in the early phase of diabetes. Indeed, an increased basal endogenous NO production accounts for the renal hyperfiltration in diabetic rats, whereas in the kidney there seems to be an enhanced nitric oxide production indicated by an increased urinary nitrate and nitrite concentration.[65,66] The cause of the increased renal nitric oxide production has not yet been elucidated. There are reports of an increased expression of inducible nitric oxide synthase (iNOS),[67] but animal studies showed that l-imino-ethyl-lysine, a specific inhibitor of iNOS, was unable to reduce the glomerular hyperfiltration.[68] Furthermore NO was not increased in normoalbuminuric type 1 diabetic patients using the isolated forearm technique.[63]

Deficiency of NO in the vascular tree is a duration-dependent process. Therefore, late in the course of diabetes, damaged endothelial cells may loose the ability to increase NO synthesis, thus favouring a proliferative and thrombogenic milieu. It is suggested that endothelial dysfunction could be the result of hyperglycaemia-induced formation of free radicals, which inactivate NO. *In vitro*, the bioavailability of NO is reduced by AGEs, which quench NO.[36] Also, hyperglycaemia interferes with the production of cyclic guanylate monophosphate (cGMP), the second messenger of NO. Hyperglycaemia is also capable of activating protein kinase C that inhibits endothelial nitric oxide synthase (eNOS).[42]

Of interest, it was recently suggested that activation of poly(ADP-ribose) polymerase (PARP) is an important factor in the pathogenesis of endothelial dysfunction in diabetes. Inhibition of PARP by a novel PARP-inhibitor PJ34 maintained normal vascular responsiveness, despite persisting hyperglycaemia in diabetic mice.[69]

Genetic influences

In contrast to diabetic retinopathy and neuropathy, which develop in the majority of diabetic patients, only 30 to 40% of type 1 diabetic patients are at risk of developing diabetic nephropathy. In view of the observed familial clustering of diabetic nephropathy, a genetic predisposition to diabetic nephropathy has been assumed. From epidemiological studies evidence appears about genetic influences on the development of microvascular complications.[70-72] The genetic susceptibility may also explain the marked differences in incidence of microvascular complications between various races.[73]

A genetic predisposition to and a parental history of hypertension are supposed to be risk factors for the development of diabetic nephropathy.[74] The likelihood of developing diabetic nephropathy was increased in patients with an elevated sodium-lithium countertransport activity, a marker of the genetic predisposition to essential hypertension.[75]

As both hypertension and the development of diabetic nephropathy seem to be genetically determined, it is tempting to search for combined genetic markers. Genes involved in the RAS are promising candidates as the RAS plays a central role in both blood pressure regulation and renal function. Association studies linking these polymorphisms with the development of diabetic nephropathy reveal conflicting results. Many reports on ACE polymorphisms suggest a contribution of the DD polymorphism in the development of diabetic nephropathy,[76] although a recent thorough review by Kunz *et al.*[77] failed to confirm the suggested association due to methodological limitations in the original studies.

CONCLUSIONS

The pathophysiology of renal diabetic microvascular complications is now slowly being unravelled. Metabolic and haemodynamic changes interfere and TGF-β seems to play a central role in this process. There is also strong evidence that hereditary factors are essential in the

development of microvascular complications. This suggests that the development of diabetic microvascular complications is a multifactorial process in which different mechanisms are likely to operate. Elucidating the pathophysiology of microvascular complications is important for the development of appropriate prevention and treatment of these complications.

NOTE

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