Molecular mechanisms of proteinuria in diabetes

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Abstract
The epidemic of Type 2 diabetes, and the parallel rising incidence of end-stage renal disease, is progressively increasing worldwide. Kidney disease is one of the major chronic microvascular complications of diabetes, and both metabolic and haemodynamic perturbations participate in its development and progression towards end-stage renal disease. Hypertension and poor metabolic control seem to interact in causing the relentless decline in renal function seen in diabetic patients. Both high circulating glucose levels and increased glomerular capillary pressure act in conjunction in stimulating the different cellular pathways leading to kidney disease. It has been suggested that mechanical forces at the glomerular level may aggravate the metabolic insult by stimulating excessive cellular glucose uptake by up-regulating the facilitative GLUT-1 (glucose transporter-1). We propose the existence of a self-maintaining cellular mechanism whereby a haemodynamic stimulus on glomerular cells induces the up-regulation of GLUT-1, an event followed by greater glucose uptake and activation of intracellular metabolic pathways, resulting in excess TGF-β1 (transforming growth factor-β1) production. TGF-β1, one of the major pro-sclerotic cytokines in diabetic kidney disease, maintains the up-regulation of GLUT-1, perpetuating a series of cellular events that result, as their ultimate effect, in increased extracellular matrix synthesis and altered permeability of the glomerular filtration barrier. Mechanical and metabolic coupling could represent an important mechanism of injury in the diabetic kidney.

Two hits, one disease: interaction between metabolic and haemodynamic perturbations, and diabetic glomerulopathy
Hypertension is an important contributor to diabetic renal disease, and the interplay between hypertension and renal disease is complex [1,2]: renal impairment contributes to the development and severity of hypertension, whereas high BP (blood pressure) accelerates the course of renal disease. Patients with diabetes and hypertension have a higher risk of end-stage renal failure [ESRD (end-stage renal disease)] than patients with hypertension alone [3]. Raised BP is often paralleled by clinical albuminuria, the earliest manifestation of systemic microvascular damage [4]. In diabetic patients, albuminuria is an early marker of renal microvascular disease and increases the risk of CVD (cardiovascular disease) morbidity and mortality [5]. Therapeutic interventions aiming at reducing albuminuria in diabetic patients are an important goal for both cardiovascular and renal protection [6].

Haemodynamic (glomerular hypertension) and metabolic (hyperglycaemia) perturbations represent the two major determinants that contribute to the development and progression of renal disease in diabetes [7–11].

Key words: albuminuria, blood pressure, diabetes, kidney disease, proteinuria, transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF).

Abbreviations used: ang-2, angiotensin-2; BP, blood pressure; DSS rat, Dahl salt-sensitive rat; GLUT-1, glucose transport-1; MCP-1, monocyte chemoattractant protein-1; SHR, spontaneously hypertensive rat; TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor.

The description of the interaction between hypertension and hyperglycaemia in the pathophysiology of diabetic kidney disease was initially made by Hostetter et al. [12,13], who found a hyperglycaemia-mediated altered glomerular capillaries autoregulation that by reducing afferent and, to a much lesser degree, efferent arteriolar tone [12,13] resulted in higher glomerular hydraulic pressure and secondary severe glomerular lesions [12,13]. Under non-pathological conditions, vascular autoregulatory mechanisms protect the glomerular capillaries from changes in systemic arterial BP [14].

The mechanisms at the basis of hyperglycaemia-mediated disruption of capillary vasoregulation are complex. Hyperglycaemia-mediated increase in vascular nitric oxide [15] and TGF-β1 (transforming growth factor-β1) [16,17] production have been implicated in vasodilation of both afferent and efferent glomerular arterioles, the latter through the production of reactive oxygen species [18]. Hyperglycaemia also activates the local tissue RAAS (renin–angiotensin–aldosterone system) [19] by local excess production of ang-2 (angiotensin-2). In diabetes, the documented higher sensitivity of the efferent (versus the afferent) glomerular arteriole to the vasoconstrictive action of ang-2 contributes to the imbalance in arteriolar tone, which results in higher glomerular capillary pressure [17,20]. As a result, in diabetes, a disproportionate systemic pressure is transmitted to the glomerular circulation, resulting in mechanical elongation of glomerular cells and activation of the cellular mechanisms that lead to glomerular damage [21].

Importantly, ang-2 directly stimulates the production of TGF-β1 [16,17], which is one of the most important cytokines that participate in the pathogenesis of diabetic
glomerulopathy [22]. In the kidney, TGF-β1 stimulates the production of other growth factors such as VEGF (vascular endothelial growth factor) [23], TGF-β1 itself [22] and CTGF (connective tissue growth factor) [24]. The excessive production of TGF-β1 and the activation of TGF-β1-mediated pathways are involved in the steps that progressively lead to glomerulosclerosis and renal interstitial fibrosis [22,25,26].

In the experimental animal model of hypertension, such as the DSS rat (Dahl salt-sensitive rat) [27,28] or the one-kidney 5/6 nephrectomy model [29], which are specifically characterized by an impairment of glomerular capillaries vasoregulation, intraglomerular pressure results in increased glomerular extracellular matrix deposition and glomerulosclerosis [30]. By contrast, in the SHR (spontaneously hypertensive rat), glomerular damage is delayed by increased preglomerular arteriolar resistance that counteracts the effect of increased systemic BP transmission on the glomerular capillaries [31]. If preglomerular vasoregulation in the SHR is impaired by uniprenchyma or diabetes, haemodynamic perturbations result in an earlier and significant increase in albuminuria, increased TGF-β1, mesangial expansion and glomerulosclerosis [1,32].

Many of the molecules involved in the pathophysiology of glomerular capillary damage in diabetes affect the expression of the facilitative glucose transporter GLUT-1 (glucose transporter-1): for example ang-2 and TGF-β1 stimulate GLUT-1 protein expression and basal glucose uptake in mesangial cells [33–36], and studies have proposed a link between GLUT-1 up-regulation in diabetes and glomerular damage.

Seminal work from Heilig et al. [37] has shown that primary GLUT-1 overexpression in mesangial cells cultured in ‘normal’ glucose concentrations, resulted in both increased basal cellular glucose uptake and extracellular matrix protein expression. Conversely, studies in which GLUT-1 expression was inhibited in mesangial cells in vitro showed a reduction in basal glucose uptake and glucose-mediated extracellular matrix production [38].

To gain further insights into the haemodynamic-mediated glomerular lesions, we asked whether haemodynamic forces might interact with GLUT-1 expression and cellular glucose uptake.

We found that mechanical stretch applied to human mesangial cells in vitro significantly up-regulated GLUT-1 protein expression and basal glucose uptake, effects that were prevented by neutralization of the action of TGF-β1 [34].

We then studied whether GLUT-1 expression differed in an animal model of both systemic and glomerular hypertension, the DSS rat on a high-salt diet [27,28], as compared with an animal model of systemic hypertension with normal capillary pressure, namely the young SHR [31].

Hypertensive DSS rats treated with a high-salt diet showed an increase in glomerular GLUT-1 expression as compared with normotensive DSS rats [34]. By contrast, in the young SHR, GLUT-1 expression was similar to its normotensive control, the WKY (Wistar Kyoto) rat [34].

The increased glomerular GLUT-1 up-regulation in the hypertensive DSS rats was paralleled by an increase in renal TGF-β1 expression when compared with their DSS normotensive controls, whereas TGF-β1 expression was similar in the young SHR and its normotensive control [34].

The mechanical-GLUT-1-mediated increase in cellular glucose transport would activate different intracellular metabolic pathways such as the polyol and hexosamine pathway, increase the production of advanced glycation end-products, activate protein kinase C and p38 MAPK (mitogen-activated protein kinase) and promote an increase in oxidative stress [39]. These pathways have been linked to TGF-β1 up-regulation, increased glomerulosclerosis and progressive impairment of glomerular function [40–47].

Further, stretch-induced up-regulation of local ang-2 and the angiotensin type-1 receptor [48] will lead to activation of TGF-β1-mediated GLUT-1 up-regulation, thus triggering a vicious cycle resulting in higher cellular glucose uptake [33,49].

In line with these observations, studies have described a greater abundance of renal cortical GLUT-1 in streptozotocin-induced diabetic rats [50,51].

The important role of TGF-β1 as a common mediator of the haemodynamic and metabolic-mediated glomerular alterations [44,52] has clearly highlighted the importance of this cytokine in the pathophysiology of diabetic kidney disease [22], and it has been shown that any intervention that by improving metabolic control and by controlling hypertension leads to down-regulation of TGF-β1 would be crucial in the treatment of diabetic nephropathy [53,54].

Other cytokines such as MCP-1 (monocyte chemoattractant protein-1), a key inflammatory mediator in the pathogenesis of nephropathy, have also been implicated in matrix accumulation and diabetic nephropathy [55], and it is of interest to note that MCP-1 is increased in angiotensin-induced hypertension [56].

Studies in experimental animal models of diabetes in which either TGF-β1 or MCP-1 action was inhibited resulted in an amelioration of diabetes–mediated renal disease [57,58]. Despite these promising results, these agents retain parallel effects on the modulation of the immune system, which will make their future use as a potential treatment for diabetic kidney disease unlikely.

Another important mediator of the development of diabetic nephropathy is VEGF. VEGF is a growth factor that promotes endothelial cell proliferation, differentiation and survival and mediates endothelium-dependent vasodilatation and permeability [59]. In animal studies, inhibition of VEGF has been shown to have beneficial effects on diabetes–induced renal disease, which suggests that VEGF has a central role in the pathophysiology of diabetic nephropathy [60,61]. Indeed VEGF expression has been implicated in the regulation of the integrity of the glomerular filtration barrier such as disruption and thickening of the glomerular basement membrane, podocytes slit pore density and nephrin expression, all phenomena related to the increased albuminuria seen in diabetes [61].

The angiopoietin/Tie-2 receptor system has also been implicated in the pathogenesis of diabetic glomerulopathy.
Data in humans have described, in Type 2 diabetic patients, elevated circulating and angiopoietin-2 levels [64]. Indeed in vitro experiments have shown that glucose and mechanical stretch stimulates angiopoietin-2 expression in endothelial cells [65,66], providing one potential mechanism for angiopoietin-2 up-regulation in diabetic nephropathy. In experimental animal models of Type 1 diabetes, renal angiopoietin-1 and angiopoietin-2 are initially up-regulated, whereas in the long term only angiopoietin-2 expression remains elevated, causing an inversion of the normal angiopoietin-1/angiopoietin-2 ratio (where angiopoietin 1 > angiopoietin 2) seen in normal physiology [62]. Collectively, it seems that a decreased ratio of angiopoietin-1 to angiopoietin-2 might play a role in the pathophysiology of diabetic nephropathy. Recent work has shown that primary angiopoietin-2 overexpression in glomeruli results in albuminuria and nephrin down-regulation as seen in diabetic glomerulopathy [67].

Conclusion
As both metabolic and haemodynamic perturbations represent important determinants for the pathogenesis of diabetic nephropathy, a multifactorial intervention in diabetic patients is needed for the prevention and delay of renal vascular complications.

Importantly, the STENO-2 study (conducted in Type 2 diabetic patients) demonstrated that a more aggressive treatment of metabolic and haemodynamic clinical parameters resulted in a significantly lower risk of micro- and macrovascular diabetic complications [68].

Clearly, the epidemic of diabetic microvascular complications necessitates the use of multiple treatments to reduce renal risk. The goal of future treatments, therefore, is metabolic and BP control combined with maximized end-organ protection.

References
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