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COMMENTARY

Mastering a mediator: blockade of CCN-2 shows early promise in human diabetic kidney disease

Stephen M. Twigg

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Abstract In diabetes complications, CCN-2 (known originally as CTGF) has been implicated in diabetic nephropathy both as a marker and a mediator of disease. This commentary addresses CCN-2 in diabetic nephropathy, in the context of the recent publication of the first human study to inhibit CCN-2 bioactivity in diabetic kidney disease.

Keywords Connective tissue growth factor · Diabetic nephropathy · Chronic kidney disease · Humanised antibody

Abbreviations

DN	Diabetic nephropathy
GFR	Glomerular filtration rate
CKD	Chronic kidney disease

Introduction

The CCN proteins have broad effects on cellular and tissue physiology and pathology. Regulating CCN-2 bioactivity in forms of human disease characterised by fibrosis is moving into the realms of human clinical trials. It is timely that CCN-2 be reviewed, as a potential bench to bedside molecular approach to the complication in diabetes where it has been mot extensively studied: diabetic kidney disease.

S. M. Twigg (✉)
Sydney Medical School, Discipline of Medicine and the Bosch Institute, The University of Sydney,
Sydney, New South Wales 200, Australia
e-mail: stwigg@med.usyd.edu.au

S. M. Twigg
Department of Endocrinology, Royal Prince Alfred Hospital,
Camperdown, New South Wales, Australia

Diabetes mellitus

Diabetes mellitus is an increasingly common chronic non-communicable disease that causes major morbidity and premature mortality. Recent global estimates indicate its prevalence is predicted to progressively increase over subsequent decades, with number of people affected increasing from 285 million in 2010 to 429 million in 2030 (Unwin et al. 2010). The combination of an ageing population, less than optimal lifestyle (diet and sedentary behaviour) including urbanisation, and susceptibility in certain ethnic groups, has caused metabolic stress and increased prevalence of failure of pancreatic beta cells to produce adequate insulin. Insulin deficiency causes hyperglycaemia, progressively into the diabetic range. Thus type 2 diabetes is increasing in prevalence and, for reasons that are less clear, type 1 diabetes which has an autoimmune basis, is also increasing in many parts of the world (Svensson et al. 2009).

Diabetes and kidney disease

In many developed countries including the USA, diabetic nephropathy (DN) is the single commonest causes of end-stage renal failure. About 25% of all Type 1 diabetes patients (Nathan et al. 2009) and at least 20% of Type 2 diabetes patients developing some degree of DN (Lehmann and Schleicher 2000). It is associated with a greatly increased mortality with historical data showing only 10% of patients with DN being alive after 40 years compared with 70% of patients without nephropathy (Andersen et al. 1983). The annual incidence of DN peaks just before 20 years duration of diabetes and declines thereafter. Therefore given the burden of diabetic renal disease, new therapeutic approaches need to be developed for the prevention of DN as well as diagnostic markers for its early detection.

Stages of chronic kidney disease and its detection

Chronic kidney disease (CKD) has five stages (1 to 5), based on the level of renal function which is detected clinically as the glomerular filtration rate (GFR) (Mathew and Corso 2009), (Jerums et al. 2009). Many diseases such as diabetes classically cause albuminuria and overt proteinuria as well as loss of glomerular filtration. The progression from normo- to microalbuminuria defines the initiation of DN as the termed incipient nephropathy stage. In stage 1 and 2 CKD there are no current GFR markers that are used routinely clinically (Jerums et al. 2009). Later stage CKD is indicated by transition from microalbuminuria to macroalbuminuria/proteinuria and overt DN associated with deterioration of renal function and development of end-stage renal disease. With increasing CKD, the death rate rises progressively, for example in type 2 diabetes from ~1.4% annually for those with no albuminuria, to 3.0% for microalbuminuria, 4.6% for macroalbuminuria and 19.2% for end-stage renal disease (ESRD) (Adler et al. 2003).

What causes diabetic renal disease?

It has been postulated that DN occurs as a result of the interplay of metabolic, genetic and haemodynamic factors in the renal microcirculation (Cooper 1998). Clinical and experimental studies have shown that both hyperglycaemia and altered glomerular haemodynamics are important to the pathogenesis of DN (Sharma and Ziyadeh 1997) and that the progression of DN may be reduced but not prevented by strict blood glucose control and antihypertensive treatment (Lehmann and Schleicher 2000). Biochemically, effects of prolonged high ambient glucose levels in the bloodstream include changes in activation of the polyol pathway, increased PKC activity, non-enzymatic glycation of circulating matrix proteins and/or aberrant synthesis or actions of growth factors and vasomodulatory agents (Sharma and Ziyadeh 1997). In a large population study there was a decrease in the cumulative incidence of DN with improved glycaemic control (Bojestig et al. 1994). A number of landmark clinical studies have shown that controlling blood glucose in diabetes can prevent onset of the complications, especially the microvascular complications including diabetic nephropathy as shown in Type 1 diabetes (1993) and in Type 2 diabetes by the UKPDS, ACCORD and ADVANCE studies (1998), (Dluhy and McMahon 2008), (Skyler et al. 2009). In clinical practice however current anti-hyperglycaemic therapies have their limitations and safe general blood glucose targets in diabetes are at a level where elevated blood glucose will continue to occur. Hypertension, when present, markedly accelerates the progression of diabetic nephropathy (Castellino et al. 1994). Genetic variants such as the ACE genotype are thought to confer susceptibility or protection from DN, and~half of the variation is DN is thought to be related to inherited genetic variants (Freedman et al. 2007). Epigenetic regulation by elevated glucose may also play a role in so-called cellular hyperglycaemic memory (Tonna et al. 2010).

Structural changes in diabetic nephropathy

Diabetic nephropathy involves structural changes that are characterised by early hypertrophy of both glomeruli and tubules, with thickening of the glomerular and the tubular basement membrane followed by progressive accumulation of extracellular matrix (ECM), particularly in the glomerular mesangium (Schleicher and Nerlich 1996), (Ziyadeh 1993). Fibrillar collagens such as collagen-I, and -III are only detected in late glomerulosclerosis while collagen-IV and fibronectin are present in the normal mesangium and are increased earlier in diabetic nephropathy (Mason and Wahab 2003). Glomerular podocyte loss and foot process effacement are also thought to be important in the development of loss of the glomerular filtration barrier and consequent albuminuria (Wolf et al. 2005). Tubulo-interstitial injury is an important indicator of renal dysfunction (Gilbert and Cooper 1999), and pathological changes described include tubular atrophy, tubular cell hypertrophy and interstitial fibrosis. Collectively glomerulosclerosis and renal tubulo-interstitial fibrosis correlate with progressive albuminuria and loss of renal filtration function (Lehmann and Schleicher 2000), (Gilbert and Cooper 1999).

CCN-2 as a potential key mediator in diabetic nephropathy

CCN-2 is emerging as a central growth factor, matrix associated protein candidate in DN. It is implicated in causing both glomerular and tubular changes in DN.

In vitro, CCN-2 is upregulated in human (Murphy et al. 1999) and rat mesangial cells (Makino et al. 2003) exposed to high glucose, partly by a PKC and TGF-β1 dependent mechanism (Murphy et al. 1999). Induction of CCN-2 mRNA and protein by advanced glycation end-products in human dermal fibroblasts (Twigg et al. 2001) (Twigg et al. 2002c) and in human renal mesangial cells (Twigg et al. 2002a) is well documented as are CCN-2 increases by reactive oxygen species (Park et al. 2001).

CCN-2 bioactivity induces kidney fibroblast proliferation and ECM synthesis (Ito et al. 1998), (Wahab et al. 2001), (Twigg and Cooper 2004). Addition of recombinant CCN-2 to cultured mesangial cells increased the expression of ECM proteins including collagens and other matrix proteins present in DN (Murphy et al. 1999). CCN-2 may also prevent matrix degradation in diabetes: when mesangial cells were grown on a matrix in high glucose, recombinant human CCN-2 prevented degradation of the matrix and this effect was attenuated by addition of an anti-CCN-2 neutralising antibody, with CCN-2 inhibiting mesangial cell derived matrix metalloproteinases (McLennan et al. 2004). Some studies have implicated CCN-2 in causing epithelial to mesenchymal transition (EMT) in renal tubular cells in diabetes, which may then lead to genesis of new activated fibroblasts in the renal interstitium (Wahab and Mason 2006), (Burns et al. 2007). All of these effects on mesenchymal cells suggest that CCN-2 can contribute to the fibrosis occurring in glomerulosclerosis and tubulo-interstitial fibrosis.

CCN-2 was also shown to have a role in mesangial cell hypertrophy by causing cell cycle arrest (Abdel-Wahab et al. 2002). Addition of intact recombinant human CCN-2 to porcine fibroblasts (Wang et al. 2003) and human dermal fibroblasts (Twigg et al. 2002b) led to increased CCN-2 levels in a time and dose dependent manner indicating that CCN-2 can up-regulate its own expression. It is possible that dysregulated feed-forward autocrine regulation of CCN-2 may result in overexpression of CCN-2 in fibrotic conditions such as glomerulosclerosis thereby aggravating the development and progression of DN (Oemar and Luscher 1997).

CCN-2 has also been studied in the renal podocyte where it may cause apoptosis (Gruden et al. 2005), (Turk et al. 2009). CCN-2 induces pro-inflammatory cytokines (Sanchez-Lopez et al. 2009) and is a potent chemotactic factor for macrophages (Cicha et al. 2005) and it is likely to contribute to early renal inflammation that precedes overt DN (Sanchez-Lopez et al. 2009).

The cellular mechanism of action of CCN-2 is complex and to some degree cell type specific. The domains or modules of CCN-2 appear to vary in mediating its functions (de Winter et al. 2008). Documented and putative signaling mechanisms utilised by CCN-2 have recently been reviewed in detail in this journal (Mason 2009), and include the nerve growth factor receptor TrKA, Type II TGF-β R signalling, LRP-1 phosphorylation, a variety of integrins, and heparan sulphate proteoglycans. CCN-2 induces ECM partly through potentiating effects of TGF-β (Riser et al. 2000). Induction of lipid rafts clustering by CCN-2 may also be involved. Second messenger systems that may be activated broadly include MAP kinase pathways, PI3K-PKB, TGF-β-SMAD signalling through multiple mechanisms and pathways post-integrin signalling such as focal adhesion kinase. CCN-2 may also induce cytokines and by protein-protein interactions, regulate bioactivity of other growth factors such as VEGF isoforms and TGF-β (Mason 2009).

CCN-2 regulation in animal diabetic nephropathy

CCN-2 is upregulated in the STZ-diabetic rodent and in the db/db diabetic mouse glomerulus and it precedes overt glomerulosclerosis in these models (Riser et al. 2000). It is increased in renal glomeruli isolated from streptozotocin (STZ) induced diabetic rats (Wada et al. 2002). Also in rodent models of DN, CCN-2 mRNA and protein was upregulated in the early stages of diabetes, followed by increases in ECM proteins (Riser and Cortes 2001), (Makino et al. 2003). CCN-2 in diabetic nephropathy was found to be present in many cell types including glomerular mesangial cells, podocytes, parietal epithelial cells (Roestenberg et al. 2006), (Umezono et al. 2006), endothelial cells, proximal tubular cells and interstitial fibroblasts (Mason 2009).

Various studies using antihypertensive agents have shown that renal growth factor up-regulation is prevented especially by agents that inhibit the renin-angiotensin-aldosterone system. A good example linking growth factors and haemodynamic effects is the prevention of CCN-2 increase in the diabetic rodent kidney by angiotensin receptor antagonists (Liu et al. 2003). From a glucose perspective, administration of aminoguanidine to inhibit the formation of advanced glycation end-products prevented the induction of CCN-2 in diabetic rodents, and in parallel, albuminuria (Twigg et al. 2002a). In larger animal models utilising non-human primates we have reported that increase in glomerular and tubular CCN-2 protein in a baboon model of type 1 diabetes preceded and predicted development of DN as albuminuria (Thomson et al. 2008).

Studies of global inhibition of CCN-2 bioactivity have implicated CCN-2 in DN. In CCN-2 heterozygous mice rendered diabetic compared with wild type controls, and anti-CCN-2 neutralising antibody studies in diabetic rodents, the diabetes induced GBM thickening was reported to be prevented by the CCN-2 inhibition strategies (van Nieuwenhoven et al. 2005).

The most definitive evidence for a role of CCN-2 in mediating DN is shown by studies that target CCN-2 specifically in the kidney: over-expression of CCN-2 in podocytes worsens diabetic nephropathy in mice (Yokoi et al. 2008), and inhibition of CCN-2 expression in diabetes by antisense oligonucleotide administered to the kidney attenuates structural and functional changes of nephropathy in mouse models of diabetes (Guha et al. 2007).

The first human study of CCN-2 inhibition in diabetic nephropathy

The published evidence that CCN-2 is up-regulated in human DN, that it exhibits bioactivity to contribute to DN, and that inhibition studies of CCN-2 in animal models prevent DN, collectively provide strong rationale to block CCN-2 in human DN. Human clinical studies require that safety and dosing of the specific intervention, in this case the inhibitor, be addressed initially in Phase I studies prior to a formal examination of efficacy in larger appropriately powered clinical trials.

In the Phase I open-label study undertaken in this report (Adler et al. 2010), a humanised neutralising anti-CCN-2 antibody in a dose escalation was examined. This antibody, FG-3019, directed against the second domain of the CCN-2 protein, has been shown in vitro and in vivo including in rodent DN to have efficacy to neutralise CCN-2 action. FG-3019 has also previously been studied in a dose escalation protocol in human idiopathic pulmonary fibrosis. In the current trial, it was delivered in a parenteral dosing schedule with intravenous infusion each 14 days, on 4 occasions (56 days). The 24 subjects studied all had well characterised renal disease based on the presence of urinary albumin in the microalbuminuria range. The majority (79%) had type 2 diabetes.

Results showed that the dosing regimen was well tolerated overall, with 21% of subjects experiencing mild adverse events on the day of infusion. No antibody response to FG-3019 was detectable. The dosing regimen suggested saturable kinetics. Follow-up across a total 1 year did not detect evidence of safety concerns. While the study was not powered to address changes in albuminuria, urinary albumin was a pre-specified end-point. The baseline urinary albumin which was in the microalbuminuria range, was more than halved on average, which is a statistically significant change across the entire cohort ($p=0.027$ vs baseline). No graded dose-response effect of the CCN neutralising antibody was observed. Markers of tubular dysfunction showed no significant change for urinary NAC or α1microglobulin/Cr, and β2M/Cr was marginally improved compared with baseline. Urinary CCN-2 was not detectable in study subjects using a whole CCN-2 protein assay or an amino-terminal CCN-2 ELISA. Plasma amino-terminal CCN-2 was found to increase transiently consistent with a delayed clearance of CCN-2 and formation of immune complexes with FG-3019, prior to complex clearance.

The phase I trial in context

This Phase I study demonstrates positive results for effectiveness of the anti-CCN-2 antibody approach in DN. Firstly, the regimen appeared to be well tolerated in a 2nd weekly dosing regimen. Secondly, results demonstrate some efficacy in terms of a reduction in albuminuria. In the author's opinion, the positive outcomes appear to be promising enough to support development of the CCN-2 neutralising antibody in Phase II and subsequent clinical trials. It is notable that the Pharma company owning FG-3019 has reportedly commenced such studies (http://www.fibrogen.com/).

Overall, the study quality appears to be quite high. For example the primary end-points of pharmacokinetics and safety were well documented. Half-life of FG-3019 was reported using a sensitive and specific assay. Safety parameters were routinely documented and monitored by an independent committee. The baseline albuminuria was carefully measured to confirm that it was established (persistent) and the follow-up examination for albuminuria was also by repeated sampling. It is unclear if urinary albumin levels were normalised by log transformation to enable appropriate statistical testing by parametric statistical analysis, although data was presented appropriately in box-whisker plots. It is of some concern that most study subjects had type 2 rather than type 1 diabetes, in that up to 50% of people with type 2 diabetes and chronic kidney disease are reported to commonly have another main cause for the kidney disease than the diabetes (Parving et al. 2002). It may well be that the renal disease resulting in the albuminuria in the subjects with type 2 diabetes was due to multiple renal pathologies, and any beneficial effect may not have been specific to diabetes. It is however somewhat reassuring that the majority of subjects had a diabetes duration of more than 10 years suggesting that the exposure to elevated blood glucose had been prolonged in most. Also, most were taking ACEI or ARB therapy, which is appropriate to current standards of clinical care. While a potential confounder of change in blood glucose appears to have been negated as HbA1c levels were unchanged across the study, an observed fall in systemic blood pressure in this uncontrolled study could have mediated the fall in albuminuria, which may or may not have been due to the FG-3019 antibody.

The clinical significance of the observed improvement in albuminuria in the trial, which was quite rapid, is uncertain. In DN, progression of albuminuria is a major risk factor for development of renal failure and also cardiovascular mortality (Parving et al. 2002). Regression of albuminuria has been well documented in DN after the administration of ACE inhibitors, and albuminuria regression correlates with improved outcomes in observational studies in diabetes (Araki et al. 2008). It follows that normalisation of urinary albumin may reflect improvements in endothelial vascular dysfunction and normalisation of renal glomerular (and possibly tubular) function. However, regression of urinary albumin has not clearly been related prospectively in randomised clinical trials to renal outcomes in diabetes and more studies are required to define the potential clinical value of aiming for regression of established albuminuria to normo-albuminuria.

In subsequent clinical trials it is envisaged that methods of administration that make the antibody easier to deliver may be employed. For example, subcutaneous injection of antibody each 2 weeks is a potential dosing regimen; at the higher dose administered in the current study iv (10 mg/kg), it would be expected to be well tolerated and may show efficacy. Study of a group of subjects with macroalbuminuria as well as those with microalbuminuria would ideally be undertaken and prevention of progression of albuminuria as well as regression would be desirable end-points. The study undertaken in the current publication was originally reported in abstract form and presented in 2006 and 2007, and it is hoped that other outcome clinical trials may be reported in peer reviewed systems in a more timely manner.

CCN-2 and its possible future targeting in diabetes

It is well recognised that the majority of pharmacological therapies to treat and prevent disease do not succeed in progression from early phases to becoming part of routine clinical care. Main reasons for failure include adverse effects or lack of efficacy. In contrast, humanised neutralising antibodies are increasingly being used in clinical practice including in chronic disease such as anti-inflammatory approaches in inflammatory arthritides (Enever et al. 2009). This antibody approach reduces the risk of adverse effects compared with use of murine antibodies that are not humanised and it may also increase the chance of benefit (Isaacs 1990), (Enever et al. 2009).

To assess efficacy of CCN-2 neutralising antibody, larger adequately powered studies, at Phase II then III level will need to be undertaken, with subjects randomised to them and with placebo control and ideally a double-blind methodology. The end-points of progression of urinary albumin, and ideally change in GFR would be desirable as both are independent risk factors for end-stage renal failure and cardiovascular mortality and effects of interventions may be divergent. CCN-2 has recognised roles in normal tissue homeostasis. It will be important to determine if its inhibition systemically leads to adverse effects related to the role of CCN-2. For example in people with diabetes who are treated with anti-CCN-2 therapy it could be envisaged that any intercurrent wound such as an abrasion or laceration may be impaired in its healing. Ideally, methods to target CCN-2 in specific tissue and cell types will be developed to increase the benefit to risk ratio of regulating CCN-2.

It is recognised that biological fluids, even urine may not be specific and sensitive enough for early changes occurring in one organ to be detected (Steinke 2009). CCN-2 shows promise as a possible marker of progressive morbidity and mortality in human DN. CCN-2 as a secreted protein is detectable in biological fluids. Clinical studies have shown that CCN-2 is increased in renal tissue and in urine in evolving diabetic nephropathy. Both urinary CCN-2 excretion and plasma CCN-2 levels were elevated in patients with DN (Gilbert et al. 2003), (Riser et al. 2003), (Roestenberg et al. 2004), while treatment with an angiotensin II receptor blocker led to a decrease in urinary CCN-2 in patients with DN (Andersen et al. 2005). In a larger cross sectional study of patients with type 1 diabetes, urinary CCN-2 excretion correlated with urinary albumin excretion and inversely with glomerular filtration rate, suggesting that urinary CCN-2 is a good indicator of declining renal function (Nguyen et al. 2006). In a large prospective study of type 1 diabetic patients plasma CCN-2 was increased in patients with DN, correlated with the rate of decline in GFR and was an independent predictor of end stage renal disease (Nguyen et al. 2008). This study is supported by another large study which showed that plasma CTGF N (amino-terminal) fragment was a risk marker for both diabetic vascular and renal disease (Jaffa et al. 2008). Recent studies of administration of CCN-2 in vivo in rodents and specific inhibition of renal proximal tubular dysfunction in rodents and man have indicated that renal proximal tubular dysfunction correlates with increased urinary excretion of CCN-2 (Gerritsen et al. 2010).

Increasingly, tissue biopsies are receiving revived attention, to detect subtle, early changes in DN (Liang et al. 2009). One small study suggested that elevated CCN-2 mRNA levels in type 1 diabetes appear to predict development of DN 3 to 4 years later (Adler et al. 2002). This data is supported by the small study in diabetic baboons where DN development was predicted by renal CTGF protein assessed 5 years earlier (Thomson et al. 2008). Finally, genetic variants in the CCN-2 promoter may directly contribute to susceptibility in DN by regulating efficiency of CCN-2 induction by TGF-β dependent signalling pathways (Wang et al. 2010).

CCN-2 has been implicated in many fibrotic diseases. In diabetes, in addition to causing kidney damage, CCN-2 may be a mediator in diabetic retinopathy, and diabetic cardiomyopathy (Leask 2010). In contrast, topical CCN-2 may have a role as therapy in diabetic wound healing (Liu et al. 2007), (Thomson et al. 2010). Hopefully adequate resources will be brought to bear and preclinical and translational research will address whether regulating CCN-2 systemically, and in time, its tissue targeting, leads to improved outcomes in people with diabetes.

Declarations of potential conflict of interest Nil

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