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New and old agents in the management of diabetic nephropathy

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Abstract

Diabetic nephropathy (DN) is a long-standing complication of diabetes mellitus and is responsible for more than 40% of end stage renal disease cases in developed countries. Unfortunately, conventional renin-angiotensin-aldosterone system (RAAS) inhibitor medications only partially protect against the development and progression of DN. Moreover, RAAS inhibitors have failed as primary prevention therapy in type 1 diabetes. Thus, agents targeting alternative pathogenic mechanisms leading to DN have been intensively investigated. Promising emerging agents have targeted neurohormonal activation (alternative components of the RAAS and neprilysin inhibition), tubuloglomerular feedback mechanisms (sodium glucose co-transporter 2 inhibition and incretin-based therapy) and renal inflammation/fibrosis. The aim of this article is to review available evidence demonstrating the potential of these agents to protect and prevent progression of DN.

Introduction

Diabetic nephropathy (DN) remains a significant public health burden responsible for more than 40% of end-stage renal disease (ESRD) requiring dialysis or renal transplantation in the Western world¹. Additional costs relate to cardiovascular complications of ESRD, since dialysis patients with diabetes are at a particularly high risk of developing coronary artery disease, stroke, and peripheral arterial disease¹. Thus, it is of paramount importance to identify safe, new agents that prevent or delay the initiation and progression of DN. Unfortunately, recent renal protection trials have either failed, demonstrated harm or reported effects that are far below expectations based on data from experimental models. For example, within the last 5 years, trials with early renin-angiotensin-aldosterone system (RAAS) blockade in patients with type 1 (T1D) and type 2 diabetes (T2D), with dual RAAS

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blockade, protein kinase C-beta inhibition, endothelin receptor antagonists and the anti-oxidant bardoxolone have reported disappointing results². Accordingly, this review will summarize promising novel therapies that may slow the progression of DN by targeting pathogenic mechanisms such as neurohormonal activation, tubuloglomerular feedback and renal inflammation/fibrosis.

Renal Protective Therapies Targeting Neurohormonal Activation

The RAAS and ACE2 Activation

Animal models, mechanistic data and extensive clinical trials support a central role for intrarenal RAAS in the development and progression of DN³. Unfortunately, RAAS inhibitor trials have also produced some disappointing results, including the failure of primary prevention studies (The Renin Angiotensin System Study [RASS]), and serious side effects observed with dual RAAS blockade². The lack of complete protection against the development of complications with traditional RAAS inhibitors underscores the need for new therapeutic strategies. Nevertheless, RAAS blockade continues to be of central importance for the management of DN due to protective effects of traditional RAAS inhibitors, and because of recent advances in novel RAAS-related pathways.

Over the last decade, new components of the RAAS have been identified, and our understanding of the processing and breakdown of angiotensins continues to evolve. In 2000, angiotensin-converting enzyme 2 (ACE2), a type 1 integral membrane protein was identified⁴. ACE2 has almost 40% homology with ACE⁴ and is especially abundant in the kidney⁵. ACE2 cleaves the C-terminal amino acid of Ang II to generate the Ang1-7 peptide, which subsequently acts via the Mas receptor to counteract the adverse effects of angiotensin (Ang) II and is thought to provide renoprotection by reducing oxidative stress, inflammation, and lipotoxicity⁵. In contrast to ACE, ACE2 activity is not responsive to conventional ACE inhibition⁵. Diabetic animal models are associated with Ang II overactivity⁶, and studies with downregulation of tubular ACE2 have been associated with accentuated albuminuria and tubular injury⁷. Additionally, ACE2-deficient mice demonstrate glomerulosclerosis⁸ and enhanced Ang II-induced renal oxidative stress with consequent renal injury⁹. Consistent with these findings, animal models have also demonstrated that increased ACE2 activity at the podocytes can attenuate the development of DN¹⁰, suggesting a potential mechanism to counteract diabetes-associated Ang II overactivity⁶. In fact, DN is associated with reduced glomerular and tubular ACE2 expression^{11,12}, and ACE2 activity is associated with glycemic control and glomerular filtration rate (GFR) in patients with DN¹³.

For these reasons, ACE2 has been investigated as a potential therapeutic target. In murine models, recombinant ACE2 lowers blood pressure and attenuates glomerular mesangial cell proliferation, oxidative stress, fibrosis and ultimately diminishes the progression of DN^{5,14}. The protective effect of recombinant ACE2 is likely due to a reduction in Ang II levels and increased in Ang 1-7 signaling, leading to reduced blood pressure, decreased NADPH oxidase activity, as well as renal histological protective effects in experimental models¹⁵. Recombinant ACE2 may provide further synergistic benefits in combination with conventional RAAS inhibition by preventing feedback escape and/or enhancing the generation of Ang 1-7, thereby augmenting vascular protective effects associated with

traditional RAAS inhibitors⁵. Finally, enhanced ACE2 bioactivity can be achieved through increased endogenous expression. The two small-molecule ACE2 activators, xanthenone (XNT) and DIZE¹⁶ increased ACE2 activity and significantly decreased blood pressure in animal models¹⁷ and correct dysfunctional vascular repair mechanisms seen in CD34+ cells isolated from diabetic individuals¹⁶. Non-peptide Mas-receptor agonists are also under investigation to determine if activation of Ang(1-7)-Mas pathways produce cardio-renal protective effects in experimental models of diabetes¹⁸. Despite the strong mechanistic rationale for ACE2 renal protection based on animal models, very little is currently known about this emerging class in humans.

Neprilysin inhibition

Another neurohormonal system strongly related to RAAS is the natriuretic peptide (NP) system (Figure 1). NPs are a family of three neurohormonal peptides: atrial, brain and c-type NPs (ANP, BNP and CNP)¹⁹. Neprilysin is a widespread enzyme responsible for degradation of NPs, and a range of other vasoactive peptides including bradykinin, substance P, Ang II and endothelin¹⁹. Neprilysin inhibitors (NEPi) lead to natriuresis, vasodilatation, reduced intraglomerular pressure and decreased proteinuria¹⁹. However, chronic isolated NEPi does not translate into clinically meaningful blood pressure reductions as the vascular effects of NEPi are often negated by upregulation of the RAAS and increased sympathetic nervous system activity²⁰. Accordingly, the beneficial renal effects of NEPi are enhanced when combined with traditional RAAS, which has led to the development of combined NEPi/RAASi agents. While no large-scale human trials have to date been conducted with NEPi or NEPi/RAASi in a chronic kidney disease (CKD) cohort, animal models have shown promising results. For instance, in a 5/6 nephrectomy model (an experimental model for CKD), AVE7688, a vaso-peptidase blocking ACE and NEP, led to increased renal synthesis of nitric oxide, decreased synthesis of endothelin-1, resulting in a significant reduction in proteinuria, glomerulosclerosis and tubulointerstitial fibrosis²¹. Similar findings have also been observed in diabetic and hypertension murine models^{22,23}. In another 5/6 nephrectomy model, omapatrilat (combined NEPi/ACEi) was associated with reduction in blood pressure, intraglomerular pressure, proteinuria and delayed progression of renal disease compared with ACEi alone²⁴, and similar protective effects have been demonstrated by others²⁵. Furthermore, LCZ696 (valsartan/sacubitril), a combined angiotensin receptor–neprilysin inhibitor provided greater reduction in cardiovascular death and hospitalization for heart failure compared to enalapril in the recent PARADIGM-HF clinic trial^{26,27}. In the PARADIGM-HF clinical trial, fewer patients in the LCZ696 group than in the enalapril group stopped their study medication due to adverse effects²⁶. The patients randomized to the LCZ696 group experienced symptomatic hypotension more frequently than patients in the enalapril group. However, cough, serum creatinine level of ≥ 2.5 mg/dL, and serum potassium >6.0 mmol/L were reported less frequently in the LCZ696 group than in the enalapril group²⁶.

In summary, ACE2 and NEP are promising therapeutic targets to supplement conventional RAAS blockade in DN. The translation of these potential therapies into clinical practice now requires investment in adequately powered clinical trials that will capture both important physiological parameters as well as long-term renal outcomes.

Renal Protective Therapies Targeting Tubuloglomerular Feedback Mechanisms

Sodium-glucose cotransporter-2 inhibitors (SGLT2i)

SGLT2i are glucose lowering agents, which are known to decrease blood pressure, weight and improve arterial stiffness^{28–32}. A more novel potential role of SGLT2i is renal protection, which is thought to be mediated by effects on the tubuloglomerular feedback mechanism (Figure 2)³². Increased filtered glucose load at the proximal tubule during hyperglycemia results in about 36% overexpression of SGLT2 in DM³². Consequently, increased reabsorption of sodium at the proximal tubule leads to decreased distal sodium delivery to the macula densa, which is incorrectly sensed as a reduction in effective circulating volume by the juxtaglomerular apparatus leading to downregulation of the tubuloglomerular feedback mechanism, vasodilation of the afferent renal arterioles and thus, hyperfiltration characteristic of DM³². The sodium transport into macula densa cells is thought to be an energy dependent process, and thus the SGLT2i-mediated decrease in sodium leads to decreased ATP breakdown to adenosine - a vasoconstrictor at the afferent renal arteriole³³, leading to reduced hyperfiltration, albuminuria and inflammation in diabetic mouse models^{34–36}. Consistent with animal data, in an 8-week add on to insulin study, empagliflozin treatment in young, uncomplicated T1D patients with hyperfiltration demonstrated a decrease in body weight, HbA1c, blood pressure and renal hyperfiltration³⁰. Similar to T1D patients, SGLT2i in T2D with CKD is associated with a decrease in estimated GFR (eGFR) and proteinuria^{37,38}. In T2D, 3 to 4 weeks of SGLT2 inhibition reduces eGFR in a dose dependent fashion, over a wide range of baseline eGFR values ranging from 30 to 90 ml/min/1.73m²³⁸. Furthermore, SGLT2 inhibition effects appear to be hemodynamically regulated as the reduction in eGFR is durable for 104-weeks of treatment and is reversed after a 3 week washout period^{35,39}. Interestingly, inhibition of SGLT2 results in lowering of plasma uric acid (PUA) levels by 10-20% in human studies^{40–42}, an effect that may be associated with additional renal protection as discussed in a separate article in this journal issue⁴³. Additionally, combination SGLT2i-ACE inhibition in T2D animal models is associated with reduced blood pressure, proteinuria, glomerular injury and renal fibrosis effects that are additive compared to either drug class alone⁴⁴. Thus, SGLT2 inhibition is associated with significant renal protective effects, including modest, acute declines in eGFR followed by the maintenance of stable renal function, and reduced albuminuria. Synergistic effects between SGLT2i and conventional RAAS blockade need to be studied to take advantage of the ability to suppress both tubular (SGLT2i) and neurohormonal (RAAS) factors central to hyperfiltration and development of both early and advanced DN. Dedicated renal outcome trials of SGLT2 inhibitors are currently ongoing and have the potential to change the clinical practice (Evaluation of the Effects of Canagliflozin on Renal and Cardiovascular Outcomes in Participants with Diabetic Nephropathy (CREDENCE) trial – NCT02065791).

Incretin-based therapies

Incretin-based therapies, such as glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidyl peptidase-4 inhibitors (DPP-4i), are another class of glucose lowering agents that

are gaining considerable interest as emerging renal protective agents. Endogenous GLP-1 lowers glucose by increasing pancreatic β -cell insulin secretion and inhibiting glucagon secretion. GLP-1 is rapidly degraded at the N-terminal by DPP-4 at the brush border of proximal tubules and glomerular podocytes⁴⁵. Higher expression and activity of DPP-4 and lower GLP-1 receptor (GLP-1R) expression was observed in diabetic animal studies⁴⁵. In patients with T2D, DPP-4 activity is increased⁴⁶ and correlates with higher HbA1c⁴⁷ and albuminuria⁴⁸. Consequently, GLP-1 and DPP-4i were shown to inhibit the $\text{Na}^+\text{-H}^+$ exchanger in the proximal tubule, which increases sodium excretion and triggers the tubuloglomerular feedback mechanism described above⁴⁹. In addition to effects on tubuloglomerular feedback, GLP-1R agonists and DPP-4i decrease oxidative stress, the local inflammatory response, counteract the action of Ang II, reduce albuminuria and glomerular sclerosis in diabetic animal models⁴⁵. Limited clinical data is available on the renal protective effects of incretin-based therapy. In T2D patients, DPP-4i treatment appears to limit the development and progression of microalbuminuria⁵⁰. Current studies are ongoing to examine glycemic and renal efficacy of linagliptin, a DPP-4i, in T2D patients with renal impairment (Efficacy, Safety, and Modification of Albuminuria in Type 2 Diabetes Subjects with Renal Disease with Linagliptin, the MARLINA - NCT 01792518).

Antifibrotic Agents in Diabetic Nephropathy

Whether it is neurohormonal or tubular factors that initiate the mechanisms leading to DN, the high intraglomerular pressure that results leads to activation of pro-inflammatory and pro-fibrotic pathways and eventually ESRD. Agents that interfere with inflammatory and fibrotic pathways are therefore of potential importance, and could be used as adjunctive therapies to medications that block neurohormonal and/or tubular mechanisms. Of particular interest are agents that block the formation of advanced glycation end products (AGE) and transforming growth factor beta (TGF- β).

AGE production is increased in response to intracellular hyperglycemia, and mediates renal injury via abnormal interaction with components of the extracellular matrix (ECM) and via stimulation of oxidative stress, chronic inflammation and renal fibrosis. AGE Receptors (RAGE) are expressed in mesangial cells in both diabetic rat and human models, and AGE-RAGE interactions promote oxidative stress and activation of the transcription factor NF- κ B, leading to the production of pro-inflammatory cytokines⁵¹. Blocking these interactions in diabetic mice lowers levels of inflammatory cytokines (e.g., tumor necrosis factor- α , interleukin-6)⁵¹. It has also been reported that serum AGE levels correlate with the progression of kidney damage in young adults with T1D⁵². Thus, AGEs are an attractive target for DN therapy. Pyridoxamine is one of the most compelling AGE inhibitors, which suppresses gene expression of TGF- β and serum AGE levels, thereby reducing albuminuria and development of impaired renal function in diabetic animal models⁵³. In T2D patients with proteinuria, although pyridoxamine treatment did not result in significant changes in serum creatinine, secondary analyses revealed that among patients with the best preservation of kidney function, pyridoxamine treatment was associated with a smaller change in baseline creatinine compared to controls⁵⁴. Thus, pyridoxamine might benefit patients with better baseline preservation of renal function. Based on these results, a phase III trial testing the safety and efficacy of pyridoxamine in T2D patients with milder DN is currently ongoing⁵⁵.

Other promising anti-inflammatory agents are being examined in patients, including the small-molecule inhibitor of Janus kinases 1 and 2 baricitinib, which was reported to reduce albuminuria, urinary levels of IP-10 and plasma levels of tumour necrosis factor-receptor 2 after treatment for 6 months⁵⁶. Further work is required to determine if these effects on translate into preservation of renal function over time.

Kidney fibrosis is a critical determinant of DN progression, and the role of TGF- β in this process has been extensively studied. Following the discovery that TGF- β expression was increased in both murine models of diabetes and human diabetes, TGF- β has been implicated as a mediator of Ang II-induced hypertrophy of mouse proximal tubular cell lines and increased mesangial collagen production in the context of hyperglycemia⁵¹. TGF- β was also found to be a mediator in hyperglycemia-induced collagen production in mouse podocytes⁵⁷ via upregulation of TGF- β receptor production and signaling⁵⁸. In humans, glomerular TGF- β 1 expression was detectable after the onset of overt DN⁵⁹. Accordingly, anti-TGF- β antibodies have been studied to determine their suitability in DN therapy. In murine diabetes models, the anti-TGF- β monoclonal antibody 1D11 was shown to reduce interstitial volume expansion when used in combination with an ACE inhibitor, compared to ACE inhibitor alone⁶⁰, and the TGF- β 1 receptor inhibitor GW788388 was found to significantly attenuate glomerulosclerosis in mice⁶¹. Unfortunately, a phase II trial of LY2382770, a human monoclonal anti-TGF- β 1 antibody, was terminated early due to lack of efficacy; LY2382770 treatment in patients with type 1 or 2 diabetes and advanced DN on background RAS inhibitor therapy appeared safe, but did not show significant additional benefit⁶².

Aside from drugs directly targeting TGF- β , pirfenidone and fluorofenidone are compounds exerting antifibrotic effects via reducing TGF- β expression^{63,64}. Clinically, combining pirfenidone and ACEi/ARB in patients with DN has been shown to better preserve eGFR compared to placebo⁶⁵. While promising, existing trials are small and larger clinical trials are necessary to test the safety and efficacy of pirfenidone as a potential DN therapy. Fluorofenidone was shown to significantly reduce TGF- β expression and collagen production *in vitro*⁶⁴. In rat models of kidney fibrosis, fluoroferidone treatment resulted in less interstitial collagen deposition compared to controls⁶⁶. Thus, although no studies have examined fluoroferidone treatment in animal models of DN, promising antifibrotic properties provide a compelling rationale to test fluoroferidone as a novel component of DN management.

Research into antifibrotic compounds in DN management has been underway for more than a decade, and interesting results are emerging from human clinical trials. Large, well-designed clinical trials are necessary to determine whether these new drugs will play an important role in the treatment of DN.

Conclusions

Despite the increasing incidence of DN complications leading to ESRD, RAAS blockade, in conjunction with glycemic control, weight loss and blood pressure control, has been the mainstay of therapy for the past 30 years. Agents targeting alternative pathogenic

mechanisms to slow or prevent DN progression include ACE2 modulators, NEPi, SGLT2 and DPP-4, as well as agents targeting renal inflammation and fibrosis (AGEs and TGF- β). The renal protective effects of UA lowering are also being investigated in the PERL study, as discussed in a separate review in this issue. Dedicated renal outcome trials with these agents are ongoing and have the potential to significantly impact current clinical practice.

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Figure 1.
The Interrelated Pathways of ACE2 and Neprilysin

Figure 2.

Tubuloglomerular feedback (TGF) and sodium glucose co-transporter 2 (SGLT 2) in (a) normal physiology, (b) hyperfiltration in early stages of diabetic nephropathy and (c) during SGLT 2 inhibition. Reproduced with permission²⁸. GFR – glomerular filtration rate.