

New therapeutic agents for diabetic kidney disease

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Diabetic kidney disease continues to be the leading cause of end-stage renal disease despite therapies targeted towards glycemic and blood pressure control, and drugs that block the renin–angiotensin–aldosterone system. New therapeutic agents are being studied to retard the progression of kidney damage due to diabetes. Glycosaminoglycans (e.g., sulodexide) target the glomerular basement membrane and have been shown to reduce albuminuria in multiple Phase II clinical trials. Inhibitors of advanced glycation end-product formation (e.g., pyridoxamine) reduce the renal accumulation of these pathogenic substrates with mixed clinical efficacy results. Protein kinase C inhibitors (e.g., ruboxistaurin) reduce renal damage from overexpression of this kinase, and have shown encouraging results in Phase II studies. Iron chelators, as antioxidant agents, work in reducing cellular damage by reactive oxygen species that are part of common final pathways in diabetic kidney disease. Other agents discussed in this review target multiple molecular pathways in diabetic kidney disease. If successfully developed, future clinical trials will evaluate the efficacy of these new drugs in slowing the progression of diabetic kidney disease.

Diabetes mellitus (DM) is a growing public health problem, with a worldwide prevalence estimated at 171 million (2.8%) in 2000 [1]. The number of patients diagnosed with DM continues to increase owing to the rise in obesity, population growth and aging [1]. The majority of diabetic patients (up to 75%) have Type 2 DM and concurrent hypertension, which is directly related to excess adiposity [2]. The increase in DM has been associated with a rise in the prevalence of diabetic chronic kidney disease (CKD) [3].

The term ‘diabetic CKD’ has been proposed by the National Kidney Foundation to replace ‘diabetic nephropathy’ in defining kidney disease caused by DM [4], and will be used in this review. Microvascular complications of diabetes involving the kidney are present in up to 40% of Type 1 and 2 diabetic patients [5]. The earliest manifestation of diabetic CKD is elevated urinary albumin excretion, as indicated by microalbuminuria (30–300 mg/24 h, or random urine albumin:creatinine ratio [ACR] 30–300 mg/g), and that, with progression in 2–3% per year [6], eventually manifests as macroalbuminuria (>300 mg/24 h or ACR >300 mg/g). These changes are usually followed by a steady decline in glomerular filtration rate. The presence of microalbuminuria increases the risk for progression to macroalbuminuria [7]. Macroalbuminuria is associated with a faster progression of kidney damage to end-

stage renal disease (ESRD) and increases the risk for cardiovascular events [8]. Diabetic CKD combined with hypertension is the leading cause of ESRD in the USA, accounting for 44% of new cases of ESRD [9]. Diabetes-related ESRD patients on hemodialysis have higher morbidity and mortality than nondiabetic patients with ESRD, and less than a third of diabetic patients survive beyond 5 years of dialysis [9].

Current management strategies for diabetic CKD are specifically targeted at aggressive glycemic and blood pressure control. Intensive multifactorial therapies directed at hyperglycemia, hypertension, microalbuminuria and hyperlipidemia in DM patients reduce albuminuria, and this translates to a reduction in cardiovascular events by approximately 50% [10]. There is a growing consensus that albuminuria should be kept as low as possible and may be a target for treatment [11]. Blockade of the renin–angiotensin–aldosterone system (RAAS) is central to the management of diabetic CKD. Angiotensin-converting enzyme inhibitors (ACEIs) in Type 1 diabetics have been shown in the Collaborative Study Group trial (using captopril) to reduce the risk of doubling of creatinine (by 48%), death and progression to dialysis (by 50%) independent of the change in blood pressure [12]. Similar benefits have been demonstrated with angiotensin receptor blockers (ARBs) in Type 2 diabetics, where the reduction in risk of doubling of creatinine and

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progression to ESRD was 33 and 23% with irbesartan in the Collaborative Study Group trial [13], and 25 and 28% with losartan in the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) study, respectively [14]. Besides lowering blood pressure, these drugs reduce albuminuria by a greater extent than other antihypertensive agents and also have cardioprotective effects [11]. Aldosterone receptor blockers have been shown to reduce blood pressure and albuminuria in diabetic CKD [15]. In addition, direct renin inhibitors reduce blood pressure by blocking the RAAS more completely, and their efficacy in diabetic CKD is being evaluated [16]. Dietary changes and lifestyle interventions, such as protein restriction [17], fish oil [18], soy protein [19] and smoking cessation [20], have shown slight benefit in diabetic CKD. Despite use of the above therapies, a large number of diabetic patients still progress to ESRD if they do not succumb to a cardiovascular event during the progression of renal disease [9]. The significant cardiovascular mortality and socio-economic burden of ESRD due to diabetes has prompted the search for newer therapeutic agents for diabetic CKD.

Pathophysiology of diabetic CKD

Microvascular injury to the glomerulus in diabetic CKD is manifested in anatomic and functional changes. The early renal changes are microalbuminuria, glomerular hyperfiltration, glomerular hypertrophy, thickening of the glomerular basement membrane and mesangial expansion due to extracellular matrix accumulation of proteins such as collagen, fibronectin and laminin. Further damage causes advanced renal changes that include proteinuria and decline in renal function due to glomerulosclerosis, glomerular arteriolar hyalinosis and tubulointerstitial fibrosis [21]. The superimposed role of systemic endothelial dysfunction and subclinical atherosclerosis on the glomerulus and renal vasculature is unknown [22]. Given the very close relationship between CKD and systemic atherosclerosis, it is believed that endothelial dysfunction in some way contributes to the pathogenesis of diabetic CKD [23].

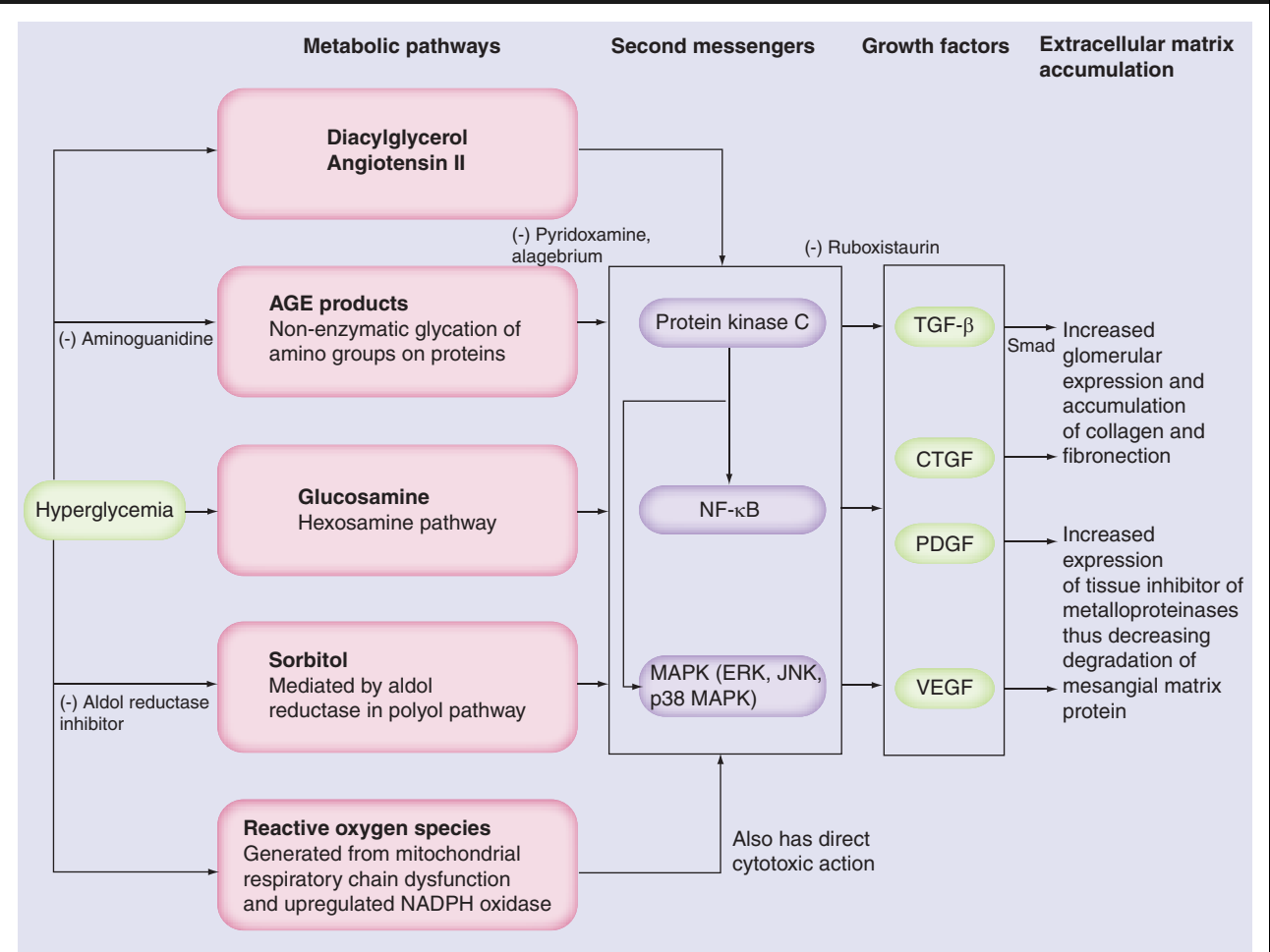
The pathophysiologic changes in diabetic CKD are a result of the interaction between metabolic and hemodynamic factors [24]. The presence of hyperglycemia impairs autoregulation within the glomerulus, thus reducing afferent arteriolar tone. The systemic pressure thus gets transmitted to the glomerulus, leading to higher glomerular

capillary pressure (glomerular hypertension) and glomerular hyperfiltration [25]. This results in damage to the glomerular capillary structure and is indicated by microalbuminuria. Hyperglycemia disrupts the glomerular autoregulation by increased production of nitric oxide, transforming growth factor- β (TGF- β) and intra-renal angiotensin II [25]. Glomerular hyperfiltration can be reversed to a large extent by tight glycemic and blood pressure control, and use of RAAS blocking agents [25].

At the molecular level, the manifestations of diabetic CKD are a consequence of the activation of certain glucose-dependent pathways that include formation of reactive oxygen species (ROS) and advanced glycation end products (AGEs), the aldose reductase/polyol pathway and the glucosamine pathway (Figure 1) [26]. These metabolic pathways activate intracellular second messengers such as protein kinase C (PKC), nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinase (MAPK), thus leading to increased expression of TGF- β , connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Prominent among these is TGF- β , as it promotes glomerular hypertrophy and stimulates extracellular matrix accumulation, the two pathologic hallmarks of diabetic CKD [27]. These growth factors mediate the renal hypertrophy, glomerulosclerosis and tubulointerstitial fibrosis of diabetic CKD. Similar mechanisms of organ damage in diabetes have been proposed to cause diabetic retinopathy [28] and atherosclerosis [29].

Diabetic CKD is a disease with multiple injury pathways and a complex pathogenesis. Oxidative stress, which is mediated in part by mobilization of iron from the lysosomes of renal tubular cells, plays a part in the pathophysiology of kidney disease [30]. This is based on the principles with which iron is reversibly oxidized and reduced. While iron is essential for its metabolic functions, it is potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as hydroxyl radical. Oxygen normally accepts four electrons and is converted directly to water. However, partial reduction of oxygen can and does occur in biological systems. Thus, the sequential reduction of oxygen along the univalent pathway leads to the generation of superoxide anion, hydrogen peroxide, hydroxyl radical and water. These free radicals, it is believed, are part of both acute and chronic mechanisms of cell injury and death within the kidney, particularly in diabetics.

Figure 1. Proposed mechanisms by which hyperglycemia causes increased extracellular matrix accumulation by mesangial cells and tubular cells.



There may be overlap between metabolic pathways, as AGEs can form reactive oxygen species, which can in turn increase AGEs. AGEs activate NF-κB, which can further activate receptors for AGE.

AGE: Advanced glycation end product; CTGF: Connective tissue growth factor; ERK: Extracellular signal-related kinase; MAPK: Mitogen-activated protein kinases; NF-κB: Nuclear factor-κB; PDGF: Platelet-derived growth factor; PKC: Protein kinase C; ROS: Reactive oxygen species; TGF-β: Transforming growth factor-β; VEGF: Vascular endothelial growth factor.

It thus seems that targeting multiple points in the altered metabolism in diabetic CKD would be more successful than a single approach in attenuating the development of proteinuria and ESRD. Current research is focused on exploring the molecular mechanisms that lead to initiation and progression of renal damage in diabetes. Understanding these pathways facilitates research on new and innovative therapeutic targets. Our review will concentrate on three important drug classes that have shown good safety profiles and have completed major Phase II clinical trials:

- Glycosaminoglycans (Table 1)
- AGE inhibitors (Table 2)
- PKC inhibitors

We also provide a brief outline of new and upcoming agents in development, including free-iron chelators (deferiprone), that may show promise in the near future (Table 3). We have graded the quality of clinical studies as prospective, randomized, controlled trial (A), intervention trial, but not randomized or blinded (B), and observational data (C).

Glycosaminoglycans Background

Glycosaminoglycans (GAGs) are important determinants of glomerular basement membrane charge-selective permeability owing to their anionic properties [31]. According to the Steno hypothesis, DM patients are susceptible to kidney

Table 1. Selected Phase II clinical trials of sulodexide in human diabetic kidney disease (in chronological order).

Study	Type of diabetic chronic kidney disease	Study size	Regimen	Duration	Reduction in albuminuria	Quality	Ref.
Velussi <i>et al.</i> (1996)	Type 2; microalbuminuria	24	p.o. sulodexide 100 mg/day	6 months	70 to 56 mg/24 h; $p < 0.01$	B	[80]
Dedov <i>et al.</i> (1997)	Type 1; micro- and macro-albuminuria	36	i.m. sulodexide 60 mg/day	3 weeks	Reduced AER ($p < 0.01$ in microalbuminuria group)	A	[81]
Sorrenti <i>et al.</i> (1997)	Type 2; micro- and macro-albuminuria	15	i.m. sulodexide 60 mg/day	4 weeks	78 to 39 $\mu\text{g}/\text{min}$ microalbuminuria group; 459 to 268 $\mu\text{g}/\text{min}$ macroalbuminuria group; $p < 0.05$	B	[82]
Poplawska <i>et al.</i> (1997)	Type 1; micro- and macro-albuminuria	14	i.m. sulodexide 60 mg/day for 10 days then p.o. 50 mg/day	1 month	349 to 91 mg/24 h; $p < 0.001$	B	[83]
Skrha <i>et al.</i> (1997)	Type 1 and 2; micro- and macro-albuminuria	53	p.o. sulodexide 60 mg/day	3 weeks	248 to 162 $\mu\text{g}/\text{min}$; $p < 0.001$	B	[84]
Solini <i>et al.</i> (1997)	Type 2; microalbuminuria	12	p.o. sulodexide 100 mg/day	4 months	128 $\mu\text{g}/\text{min}$ to 39 $\mu\text{g}/\text{min}$; $p = 0.03$	A	[85]
Szelanowska <i>et al.</i> (1997)	Type 1; microalbuminuria	15	i.m. sulodexide 60 mg/day	3 weeks	95 $\mu\text{g}/\text{min}$ to 39 $\mu\text{g}/\text{min}$; $p < 0.01$	B	[86]
Gambaro <i>et al.</i> (2002)	Type 1 and 2; micro- and macro-albuminuria	223	p.o. sulodexide 50, 100 and 200 mg/day	4 months	log AER 5.2 to 4.1 in 200 mg/day group; $p < 0.05$	A	[87]
Achour <i>et al.</i> (2005)	Type 1 and 2; micro- and macro-albuminuria	60	p.o. sulodexide 50 mg/day	1 year	260% reduction; $p < 0.001$	B	[88]

Quality A: Prospective, randomized, controlled trial; Quality B: Intervention trial, but not randomized or blinded.
AER: Albumin excretion rate; i.m.: Intramuscular; p.o.: Per os.

damage because of a defect in the content and sulfation of GAGs that is worsened by hyperglycemia [31]. The reduced GAG content and under-sulfation have been proposed to lead to albuminuria and progressive kidney damage. In addition, heparanase, an enzyme that degrades heparan sulfate, is upregulated in glomerular epithelial cells in response to hyperglycemia [32]. Thus, GAGs have been proposed to act by:

- Restoring the anionic GAG charges on the glomerular basement membrane;
- Inhibiting TGF- β [33];
- Inhibiting heparanase, thus allowing reconstruction of heparan sulfate content and restoration of basement-membrane charge selectivity [34].

Correcting these abnormalities in GAGs with exogenous administration of GAGs is a reasonable therapeutic target in diabetic CKD [35].

Properties

Glycosaminoglycans include a broad category of molecules – heparin, low-molecular-weight heparin, heparan sulfate, dermatan sulfate and mixed formulations, such as sulodexide and danaparoid sodium. Among these, sulodexide has been the most studied because it is available in an oral formulation. Sulodexide contains a naturally occurring GAG extracted from porcine intestinal mucosa, and contains a fast-moving heparan-like fraction (80%), as well as a dermatan sulfate fraction (20%) [36]. Sulodexide differs from other GAGs, such as heparin, in having a longer half-life and a reduced effect on systemic rheostasis.

Animal studies

Animal studies first suggested the potential use of GAGs when diabetic mice and rats were found to have reduced synthesis of GAGs, both

Table 2. Advanced glycation end products inhibitors.

Compound	Mechanism of action/study findings	Ref.
Aminoguanidine	Inhibits AGE formation by scavenging advanced glycation intermediates	
Animal studies	STZ diabetic rats treated with aminoguanidine for 32 weeks had a lower rise in albuminuria and reduction in AGE accumulation and mesangial expansion [89]	[89]
	OLETF rats treated with aminoguanidine for 40 weeks had reduction in serum AGE, albuminuria, mesangial volume and glomerular basement-membrane thickness [90]	[90]
Clinical trials	STZ diabetic rats treated with aminoguanidine for 6 months had a lower expression of TGF- β , PDGF and type IV collagen accumulation [91]	[91]
	Randomized, placebo-controlled trial (ACTION I) in 690 patients with Type 1 diabetes mellitus and macroalbuminuria (>500 mg/g) randomized to pimeganide for 2–4 years or placebo showed a greater reduction in proteinuria (-732 mg/24 h in the low-dose group and -329 mg/24 h in the high-dose group vs -35 mg/24 h in the placebo group; $p < 0.001$) and a trend for lower risk of doubling of creatinine (20% in treated group vs 26% in placebo group; $p = 0.099$) (A) [92]	[92]
	A similar study in Type 2 diabetics (ACTION II) was terminated early owing to safety concerns and apparent lack of efficacy [93]	[93]
ALT-711 (alagebrium)	Breaks AGE crosslinks	
Animal studies	STZ diabetic rats treated with ALT-711 for 16–32 weeks had lower development of albuminuria, a reduction in renal AGE accumulation, renal hypertrophy, and decreased expression of TGF- β , connective tissue growth factor and collagen [94]	[94]
	STZ diabetic rats treated with ALT-711 for 16–32 weeks had reduced expression of PKC- α , VEGF, fibronectin and laminin and lower albuminuria [95]	[95]
	db/db diabetic mice treated with ALT-711 for 12 weeks had serum AGE, albuminuria, glomerular hypertrophy and basement-membrane thickening [96]	[96]
Clinical trials	A Phase II clinical trial is being planned to study the efficacy of alagebrium in Type 1 diabetics with microalbuminuria (NCT00557518) [205]	[205]
Soluble receptor for AGE	Competitively blocks the cellular receptors for AGEs	
Animal studies	STZ diabetic rats treated with sRAGE reduced vascular hyperpermeability in the kidney [97]	[97]
	STZ diabetic mice treated with sRAGE for 6 weeks had reduced expression of TGF- β and accumulation of fibronectin and α IV collagen [98]	[98]
	STZ diabetic mice treated with RAGE antibodies for 2 months had reduced albuminuria and an increase in basement-membrane thickness [99]	[99]
	Obese db/db mice treated with RAGE antibodies for 2 months had reduced changes in glomerular volume and albuminuria and normalized the changes in basement-membrane thickness [100]	[100]
Clinical trials	A Phase II clinical trial is recruiting patients to study the effect of TTP-488 RAGE inhibitor on Type 2 diabetes and persistent albuminuria (NCT00287183) [206]	[206]
Benfotiamine	Transketolase activator that directs glucose substrate to pentose phosphate pathway, thus reducing AGE formation	
Animal studies	STZ diabetic rats treated with high-dose thiamine and benfotiamine for 24 weeks inhibited the development of microalbuminuria and proteinuria, inhibited diabetes-induced hyperfiltration and reduced AGE products [130]	[130]
Clinical trials	A Phase IV clinical trial is planned to study the effect of benfotiamine in reducing urinary β -2 microglobulin and albumin in patients with Type 2 diabetes and microalbuminuria (NCT00565318) [210]	[210]

A: Prospective, randomized controlled trial; ACTION: A Coronary Disease Trial Investigating Outcome with Nifedipine Gastrointestinal Therapeutic System; AGE: Advanced glycation end products; OLETF: Otsuka Long Evans Tokushima Fatty; PKC: Protein kinase C; STZ: Streptozotocin; RAGE: Receptors for advanced glycation end products; sRAGE: Soluble rAGE.

glomerular proteoglycans and basement-membrane heparan sulfate proteoglycans [37]. In rats with streptozotocin (STZ)-induced Type 1 diabetes, the use of GAGs prevented albuminuria and mesangial matrix expansion [38]. In the same study, treatment of cultured mesangial cells with GAGs suppressed the transcription of TGF- β , possibly via inhibition of its regulator PKC.

Clinical trials

Most of the clinical trials studying GAGs in diabetic CKD have used oral sulodexide (Table 1). In general, these Phase II trials, in patients with Type 1 and 2 DM, have shown that sulodexide reduces albuminuria. The majority of these studies were short term (less than 6 months) and did not study the effect of GAGs on progression of diabetic CKD. The Diabetic Nephropathy and Albuminuria Sulodexide (DiNAS) study was a randomized, double-blind, placebo-controlled multicenter European trial involving 223 patients with Type 1 and 2 DM, micro- and macro-albuminuria and serum creatinine levels of less than 150 $\mu\text{mol/l}$ ($=1.7 \text{ mg/dl}$) to determine the duration and efficacy of albuminuria reduction with escalating doses of sulodexide [39]. After 4 months of sulodexide treatment, there was significant albuminuria reduction in patients treated with 50 mg/day (30% reduction with baseline log albuminuria = 4.62; $p < 0.03$ versus placebo), 100 mg/day (49% reduction with baseline log albuminuria = 4.58; $p < 0.0001$ vs placebo) and 200 mg/day (74% reduction with baseline log albuminuria = 5.25; $p < 0.0001$ vs placebo). Furthermore, this effect was maintained for up to 8 months in patients treated with 200 mg/day sulodexide (62% reduction; $p < 0.05$ vs baseline). This benefit was seen independent of the type of diabetes (Type 1 or 2), degree of albuminuria, blood pressure control and glycemic control. Furthermore, sulodexide reduced albuminuria in patients previously receiving ACEIs.

Future clinical trials

Two double-blinded, placebo-controlled, randomized, multicenter Phase III and IV trials were designed to study the renoprotective potential of sulodexide [40]. The Sulodexide Microalbuminuria Trial examined the efficacy of sulodexide administered over 26 weeks in 1000 patients with Type 2 diabetes, hypertension and micro-albuminuria despite maximal therapy with ACEIs/ARBs, with the outcome being reversion to normoalbuminuria with 25% or greater

decrease in the urinary albumin:creatinine ratio or a 50% or greater reduction in the urinary albumin:creatinine ratio (NCT00130208) [201]. This study was completed in March 2008, and showed that sulodexide failed to meet the above end points [202]. The Sulodexide Overt Nephropathy Trial evaluated sulodexide in 2240 patients with Type 2 diabetes, hypertension and proteinuria of 900 mg/24 h or greater despite maximal therapy with ACEIs/ARBs, with the primary outcome being time to a composite end point of doubling of serum creatinine or ESRD (NCT00130312) [203]. This study has recently been terminated, as an interim analysis showed no difference to a placebo [202].

Expert commentary

Studies so far suggest that sulodexide reduces urinary excretion of albumin at the level of the glomerulus, independent of changes in blood pressure or the renin-angiotensin system. However, Phase III and IV renal outcomes trials of sulodexide failed to show this benefit beyond maximal doses of ACEIs/ARBs. The effect of sulodexide on other organs is not known – although it may exert endothelium-protective effects in endothelial dysfunction [41]. Additional studies evaluating cardiovascular end points [42], and adverse effects including rheostatic changes [43], will be called for if this drug is approved for a renal indication.

Advanced glycation end product inhibitors

Background

Advanced glycation end products are complex, irreversible sugar modifications of proteins and lipoproteins [44]. In the presence of hyperglycemia, non-enzymatic glycation of proteins leads to the formation of Amadori and Maillard intermediate products, which then undergo oxidative decomposition, leading to increased production of pathogenic AGEs such as N ϵ -(carboxymethyl)lysine, N ϵ -(carboxyethyl)lysine and pentosidine [44]. AGEs produce structural alterations in the basement membrane and extracellular matrix by increasing expression of type IV collagen and by forming crosslinks between the extracellular matrix proteins, resulting in a change in surface charge and membrane permeability. The receptors for AGEs (RAGEs) are found in the renal tubular epithelial cells, mesangial cells and podocytes [45]. In addition, RAGE is expressed on T lymphocytes, monocytes and macrophages, and plays a role in

Table 3. New therapeutic agents for diabetic chronic kidney disease .

Compound	Mechanism of action and study findings	Quality	Ref.
Pirfenidone	Anti-fibrotic and anti-inflammatory agent that inhibits TGF-β and NADPH oxidase		
Animal studies	STZ diabetic rats treated with 4 weeks of pirfenidone showed reduction in renal collagen deposition and fibronectin, thus reversing renal fibrosis		[101]
Clinical trials	Open-label study in 18 patients with refractory FSGS (baseline GFR = 26 ml/min/1.73 m ²) showed slowing in the GFR decline from -0.61 to -0.25 ml/min/1.73 m ² , with no change in proteinuria (baseline proteinuria = 3.37 g/day)	B	[102]
Future clinical studies	Phase I and II clinical trial to assess effect of pirfenidone on GFR in Type 1 and 2 diabetic patients with advanced kidney disease (GFR: 20–75) (NCT00063583)		[207]
Thiazolidinediones (PPAR-γ agonists)	Unclear, but maybe due to inhibition of TGF-β and genes involved in collagen/fibronectin formation and reduced serum/renal interstitial TNF-α		
Animal studies	Obese hypertensive Type 2 diabetic rat model treated with 26 weeks of pioglitazone showed reduction in proteinuria, glomerulosclerosis and TGF- β		[103]
	STZ diabetic rats treated with 8 weeks of pioglitazone showed reduction in albuminuria, glomerular hypertrophy and suppressed expression of TGF- β		[104]
Clinical trials	Randomized, placebo-controlled study in 29 Type 2 diabetic patients and microalbuminuria randomized to 12 weeks 8 mg/day rosiglitazone or placebo showed greater reduction in albuminuria in the rosiglitazone group vs placebo (-8.6 vs +3.4 mg/24 h; p < 0.05)	A	[105]
	Randomized, open-label study in 30 patients with Type 2 diabetes and microalbuminuria randomized to 12-week 400 mg/day troglitazone or 500 mg/day metformin showed significant reduction in albuminuria in the troglitazone group (70 to 40 mg/g; p = 0.021) vs no change in the metformin group	B	[106]
	Open-label study in patients with Type 2 diabetes and microalbuminuria evaluated for cardiac safety randomized to 52 week 4 mg twice daily rosiglitazone vs glyburide showed 43% of patients achieving normoalbuminuria in the rosiglitazone group vs 6% in the glyburide group (p = 0.51)	C	[107]
	Randomized, controlled trial with 60 Type 2 diabetic patients, macroalbuminuria and CKD (3 or 4) randomized to 12 months 100 mg/day losartan + 30 mg/day pioglitazone vs 100 mg/day losartan showed greater reduction in proteinuria (2.6 to 1.3 g/l) and slower decline in GFR in the pioglitazone group vs the losartan alone group (2.2 to 1.6 g/l)	B	[108]
Future clinical studies	Randomized, double-blind, placebo-controlled study that investigated the effect of rosiglitazone on renal plasma flow, GFR and albuminuria in Type 2 diabetic patients with a GFR of 30–70 and macroalbuminuria (NCT00324675)		[208]
BMP-7	Reduces profibrotic action of TGF-β and preserves podocyte integrity		
Animal studies	STZ diabetic rats treated with 16 weeks of BMP-7 partially reversed diabetic-induced kidney hypertrophy, restored GFR and albuminuria and prevented glomerulosclerosis		[109]
Clinical trials	STZ diabetic mice treated with 5 months of BMP-7 inhibited tubular inflammation and tubulointerstitial fibrosis		[110]
Future clinical studies	None		
	None		

Quality A: Prospective, randomized, controlled trial; Quality B: Intervention trial, but not randomized or blinded; Quality C: Observational data.

ACEi: ACE inhibitor; AGE: Advanced glycation end products; ALTITUDE: Aliskiren Trial in Type 2 Diabetes Using Cardiovascular and Renal Disease Endpoints;

ARB: Angiotensin receptor blocker; CKD: Chronic kidney disease; CTGF: Connective tissue growth factor; DM: Diabetes mellitus; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease;

FSGS: Focal segmental glomerulosclerosis; GFR: Glomerular filtration rate; PKC: Protein kinase C; ROS: Reactive oxygen species; STZ: Streptozotocin.

Table 3. New therapeutic agents for diabetic chronic kidney disease (cont.).

Compound	Mechanism of action and study findings	Quality	Ref.
Anti-CTGF agents	Block the matrix accumulation and renal fibrosis due to CTGF		
Animal studies	STZ diabetic rats and diabetic db/db mice treated with CTGF-ASO reduced serum creatinine, albuminuria and progression of fibrosis		[111]
Clinical trials	Obese db/db Type 2 diabetic mice treated with neutralizing CTGF monoclonal antibody (FG-3019) reduced albuminuria, hyperfiltration and basement-membrane thickening		[112]
Future clinical studies	Open-label Phase I study of treatment with 42 days of FG-3019 in 20 patients with Type 1 or 2 diabetes and microalbuminuria showed reduction in albuminuria from 48 to 29 mg/g	B	[113]
	None		
PPAR-α agonists	Unclear; likely due to both indirect metabolic effects and direct renal effect		
Animal studies	Type II diabetic db/db mice treated with 8 weeks of fenofibrate decreased albuminuria, glomerular hypertrophy and mesangial matrix accumulation		[114]
Clinical trials	STZ diabetic rats treated with 8 weeks fenofibrate suppressed plasminogen activator inhibitor 1 and TGF- β expression and reduced extracellular matrix deposition		[115]
Future clinical studies	Open-label study in 314 patients with Type 2 diabetes evaluated for progression of atherosclerosis randomized to 38 months fenofibrate vs placebo had a reduced proportion of patients progressing from normo- to micro-albuminuria in the fenofibrate group (2.9%) compared with the placebo group (17.7%; $p < 0.001$).	C	[116]
	None		
(Pro)renin receptor blocker	Binds to prorenin receptor, thus inhibiting further angiotensin production and activation of extracellular regulated kinases (which regulate TGF-β activity)		
Animal studies	STZ diabetic rats treated with 12 weeks of (pro)renin receptor blocker showed no worsening of proteinuria or glomerulosclerosis		[117]
Clinical trials	STZ diabetic AngII type 1a receptor gene-deficient mice treated with a prorenin blocker prevented proteinuria and glomerulosclerosis and inhibited the extracellular receptor kinase activity		[118]
Future clinical studies	None		
	None		
MMP	Hyperglycemia downregulates MMPs in the glomerulus, thus suppressing extracellular matrix degradation and leading to mesangial expansion		
Animal studies	STZ diabetic mice treated with 4 weeks of MMP-1 gene delivery by microspheres implanted under the renal capsule reduced collagen content and blood urea nitrogen		[119]
Clinical trials	None		
Future clinical studies	Phase I clinical trial studying the relation of glycemic control with different levels of MMP activity in Type 1 diabetics without kidney damage (NCT00067886)		[209]

Quality A: Prospective, randomized, controlled trial; Quality B: Intervention trial, but not randomized or blinded; Quality C: Observational data.

ACEi: ACE inhibitor; AGE: Advanced glycation end products; ALTIITUDE: Aliskiren Trial in Type 2 Diabetes Using Cardiovascular and Renal Disease Endpoints;

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Table 3. New therapeutic agents for diabetic chronic kidney disease (cont.).

Compound	Mechanism of action and study findings	Quality	Ref.
Endothelin receptor antagonist	Binds to endothelin receptor, thus blocking the profibrotic and vasoconstrictive properties of endothelin.		
Animal studies	STZ diabetic rats treated with 10 weeks ABT-627 reduced albuminuria by inhibiting renal inflammation and TGF- β production [120]		[120]
	STZ diabetic rats treated with 1 month bosentan prevented increase in urinary excretion of protein, collagen I, fibronectin and TGF- β [121]		[121]
	STZ diabetic rats treated with 36 weeks endothelin receptor antagonists reduced proteinuria by 50% and normalized the glomerular matrix deposition of fibronectin and collagen IV [122]		[122]
Clinical trials	A randomized, placebo-controlled, double-blind Phase IIb study in 286 patients with Type 2 diabetes and macroalbuminuria treated with 12 weeks escalating dose of SPP301 showed a 30% reduction in albuminuria. Phase III study terminated due to increased peripheral edema A [123]	A	[123]
Future clinical studies	None		
Aldose reductase inhibitor	Inhibits PKC activity and the enhanced production of TGF-β and extracellular matrix proteins in diabetic kidney disease		
Animal studies	STZ diabetic rats treated with 6 months tolrestat reduced progression of albuminuria and retinal basement-membrane thickening [124]		[124]
	STZ diabetic rats treated with 6 months epalrestat prevented mesangial expansion [125]		[125]
	Placebo-controlled study in 20 Type 1 diabetic patients and macroalbuminuria treated with 6 months 200 mg/day tolrestat showed significant reduction in albuminuria and decreases in GFR and filtration fraction [126]		[126]
Clinical trials	Case-control study in 35 Type 2 diabetic patients and microalbuminuria allocated to 5-year 150 mg/day epalrestat (cases) showed stable albuminuria (81 to 87 mg/g) and decreased progression of kidney disease (1.77 to 1.38 mg/dl) in cases vs controls (82 to 301 mg/g and 1.77 to 1.55 mg/dl, respectively). B [127]	B	[127]
Future clinical studies	None		
Vasopeptidase inhibitor	Unclear, but partly due to bradykinin β_2 receptor activation		
Animal studies	Zucker diabetic fatty rats treated with 10 weeks AVE 7688 prevented albuminuria and reduced severity of glomerulosclerosis and tubulointerstitial damage [128]		[128]
	Zucker diabetic fatty rats treated with 27 weeks AVE 7688 reduced albuminuria, improved clearance of AGE and inhibited AGE formation [129]		[129]
Clinical trials	None		
Future clinical studies	None		

Quality A: Prospective, randomized, controlled trial; Quality B: Intervention trial, but not randomized or blinded; Quality C: Observational data.

ACEi: ACE inhibitor; AGE: Advanced glycation end products; ALTTUDE: Aliskiren Trial in Type 2 Diabetes Using Cardiovascular and Renal Disease Endpoints;

ARB: Angiotensin receptor blocker; CKD: Chronic kidney disease; CTGF: Connective tissue growth factor; DM: Diabetes mellitus; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease;

FSGS: Focal segmental glomerulosclerosis; GFR: Glomerular filtration rate; PKC: Protein kinase C; ROS: Reactive oxygen species; STZ: Streptozotocin.

Table 3. New therapeutic agents for diabetic chronic kidney disease (cont.).

Compound	Mechanism of action and study findings	Quality	Ref.
N-acetylcysteine	Antioxidant. Thiol-containing radical scavenger and glutathione precursor protects cells by reducing ROS generation (oxidative stress)		
Animal studies	Renal tubular epithelial cells treated with N-acetylcysteine reduced hyperglycemia-induced cellular hypertrophy and MAPK activation	[131]	
	Cultured glomerular mesangial cells treated with high concentration glucose and N-acetylcysteine had reduced expression of plasminogen activator inhibitor-1, which plays an important role in remodeling of the extracellular matrix in glomeruli	[132]	
Clinical trials	STZ diabetic rats treated with 6 months N-acetylcysteine had reduced AGE deposition in glomeruli, and diabetes-associated increase in glomerular volume	[133]	
Future clinical studies	None performed on progression of diabetic CKD		
	Randomized, open-label trial to study the effect on albuminuria reduction with 600 mg twice-daily N-acetylcysteine treatment for 3 months in Type 2 DM patients with macroalbuminuria (>500 mg/24 h) (NCT00556465)	[211]	
Adenosine A2A agonist	Activation of adenosine A2A receptors leads to potent anti-inflammatory activity		
Animal studies	STZ diabetic rats treated with 6 weeks A2A agonist had reversal of albuminuria and reduced urinary inflammatory cytokines	[134]	
Clinical trials	None		
Future clinical studies	None		
Erythropoiesis-stimulating agents	Antiapoptotic effect and stimulation of regenerative cells		
Animal studies	db/db mice treated with low-dose continuous erythropoietin receptor activator (non-hematologically effective) had reduced glomerular and tubular TGF- β , collagen and reduced mesangial expansion. Reduction in albuminuria in this group persisted even after phlebotomy	[135]	
Clinical trials	None evaluating progression of diabetic CKD		
Future clinical studies	Randomized, double-blind, controlled trial to study the effect of darbepoietin on mortality, cardiovascular events and progression to ESRD in Type 2 DM patients with CKD (eGFR: 20–60) (Trial to Reduce Cardiovascular Events with Aranesp [darbepoetin alfa] Therapy NCT00093015)	[212]	
Renin inhibitor	Renin inhibitor blocks the renin-mediated cleavage of angiotensinogen to angiotensin I		
Animal studies	Diabetic transgenic (mRen-2) rats treated with 16 weeks aliskiren had reduced blood pressure, albuminuria, glomerulosclerosis and tubulointerstitial fibrosis	[136]	
Clinical trials	None evaluating progression of diabetic CKD		
Future clinical studies	Randomized, double-blind, placebo-controlled trial to study the effect of increasing doses of aliskiren on proteinuria in Type 2 DM patients with micro- and macro-albuminuria (NCT00464776)	[213]	
	Randomized, double-blind trial to study the renoprotective effect of aliskiren alone or in combination with irbesartan in Type 2 DM patients with micro- and macro-albuminuria (NCT00464880)	[214]	

Quality A: Prospective, randomized, controlled trial; Quality B: Intervention trial, but not randomized or blinded; Quality C: Observational data.

ACEi: ACE inhibitor; AGE: Advanced glycation end products; ALTTUDE: Aliskiren Trial in Type 2 Diabetes Using Cardiovascular and Renal Disease Endpoints;

ARB: Angiotensin receptor blocker; CKD: Chronic kidney disease; CTGF: Connective tissue growth factor; DM: Diabetes mellitus; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease;

FSGS: Focal segmental glomerulosclerosis; GFR: Glomerular filtration rate; PKC: Protein kinase C; ROS: Reactive oxygen species; STZ: Streptozotocin.

Table 3. New therapeutic agents for diabetic chronic kidney disease (cont.).

Compound	Mechanism of action and study findings	Quality	Ref.
	Open-label study to evaluate the time course of proteinuria reduction with aliskiren in Type 2 DM patients with micro- and macro-albuminuria (NCT00461136)		[215]
	Randomized, double-blind, placebo-controlled trial to study the effect of aliskiren on reduction in urine albumin:creatinine ratio in Type 2 DM patients with hypertension and proteinuria (NCT00097955)		[216]
	Randomized, double-blind, placebo controlled trial to study the effect of aliskiren on blood pressure reduction in Type 2 DM patients with hypertension with inadequate response to valsartan/hydrochlorothiazide (NCT00219102)		[217]
	Randomized, double-blind, trial to study the effect of increasing doses of aliskiren alone or in combination with ramipril on blood pressure reduction in Type 1 or 2 DM patients with hypertension (NCT00219089)		[218]
	Randomized, double-blind, placebo-controlled trial to study the effect of aliskiren on reduction in cardiovascular events in Type 2 DM patients with coronary artery disease, stroke or congestive heart failure and/or reduced kidney function (ALITUDE; NCT00549757)		[219]
1,25(OH)₂ vitamin D₃	Active vitamin D analog inhibits the renin-angiotensin system and mesangial proliferation		
Animal studies	Cultured mesangial cells treated with high-concentration glucose and 1,25 (OH) ₂ vitamin D ₃ had reduced fibronectin production, activation of renin-angiotensin system and TGF-β		[137]
	Cultured mesangial cells treated with high-concentration glucose and 1,25 (OH) ₂ vitamin D ₃ had reduced NF-κB activation and monocyte chemoattractant protein. STZ diabetic mice treated with paricalcitol had reduced glomerulosclerosis and fibronectin		[138]
Clinical trials	Three randomized, double-blind, placebo-controlled trials in 220 stage 3 and 4 CKD patients (including diabetes) with secondary hyperparathyroidism treated with paricalcitol for parathyroid hormone suppression showed 3.2-fold greater odds for proteinuria reduction than placebo	C	[139]
Future clinical studies	Randomized, double-blind, placebo-controlled trial to study the effect of cholecalciferol on albuminuria reduction in Type 2 DM patients with microalbuminuria and a GFR of >60 (NCT00552409)		[220]
	Randomized, double-blind, placebo-controlled trial to study the effect of paricalcitol on albuminuria reduction in Type 2 DM patients with a GFR of 15–90 and on stable dose of angiotensin-converting enzyme inhibitor and/or angiotensin II receptor blocker therapy (NCT00421733)		[221]
ADAM10 inhibitor	Inhibitor of ADAM, which sheds adhesion molecules and cytokines from the cell membrane, favoring leukocyte adhesion to endothelial cells		
Animal studies	Salt-sensitive rats (model of hypertension-induced proteinuria) treated with XL784 alone or in combination with lisinopril resulted in a dose-dependent reduction in proteinuria and glomerular disease		[140]
Clinical trials	Phase II randomized, double-blind, placebo-controlled trial of XL784 in patients with Type 2 DM and macroalbuminuria and a GFR of >30 ml/min/1.73 m ² on ACEi/ARB showed no significant reduction in albuminuria (baseline level = 1138 mg/g) as compared with placebo	A	[141]
Future clinical studies	None		

Quality A: Prospective, randomized, controlled trial; Quality B: Intervention trial, but not randomized or blinded; Quality C: Observational data.

ACEi: ACE inhibitor; AGE: Advanced glycation end products; ALITUDE: Aliskiren Trial in Type 2 Diabetes Using Cardiovascular and Renal Disease Endpoints;

ARB: Angiotensin receptor blocker; CKD: Chronic kidney disease; CTGF: Connective tissue growth factor; DM: Diabetes mellitus; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease;

FGS: Focal segmental glomerulosclerosis; GFR: Glomerular filtration rate; PKC: Protein kinase C; ROS: Reactive oxygen species; STZ: Streptozotocin.

Table 3. New therapeutic agents for diabetic chronic kidney disease (cont.).

Compound	Mechanism of action and study findings	Quality	Ref.
Spirinolactone	Antagonist to aldosterone that causes progression of renal disease by TGF-β activation and collagen accumulation		
Animal studies	STZ diabetic rats treated with spironolactone partially reversed the diabetic-induced glomerular hypertrophy, albuminuria, expression of TGF- β and fibronectin, and oxidative stress [142]		[142]
	STZ diabetic rats and db/db mice treated with eplerenone reversed the diabetic-induced glomerular hypertrophy, mesangial expansion, tubulointerstitial injury and TGF- β expression [143]		[143]
	Type II diabetic rats and mice treated with 8 months of spironolactone had decreased albuminuria and glomerulosclerosis, and reduced CTGF and collagen synthesis [144]		[144]
	Type II diabetic rats and mice treated with spironolactone had decreased urinary excretion of albumin, monocyte chemotactic peptide-1, reduced NF- κ B activation and macrophage infiltration [145]		[145]
Clinical trials	Open-label study in 13 patients with Type 2 DM and micro- or macro-albuminuria with aldosterone escape receiving ACEi, treated with 24-week 25 mg spironolactone showed reduction in albuminuria (389 to 233 mg/g; $p < 0.05$) [146]	B	[146]
	Open-label study in 32 patients (17 diabetic) with Type 2 DM and proteinuria (>0.5 g/day) on ACEi treated with 12-week 25 mg spironolactone showed reduction in proteinuria (1162 to 722 mg/day; $p < 0.05$) in all patients and urinary collagen in diabetics [147]	B	[147]
	Randomized, double-blinded, placebo-controlled trial in 21 patients with Type 2 DM and macroalbuminuria, despite use of ACEi/ARB, treated with 25 mg spironolactone for 8 weeks showed significantly lower albuminuria in the spironolactone group (1067 mg/g) vs the placebo group (1566 mg/g) and lower blood pressure (6/4 mmHg) as compared with placebo [148]	A	[148]
	Randomized, double-blinded, placebo-controlled trial in 20 patients with Type 2 DM and macroalbuminuria, despite use of ACEi/ARB, treated with 25 mg spironolactone for 2 months showed significant reduction in proteinuria (by 30% from baseline level of 834 mg/24 h) as compared with placebo [149]	A	[149]
	Randomized, double-blinded, placebo-controlled trial in 20 patients with Type 2 DM and nephrotic range albuminuria (2.4 g/24 h) on long-term ACEi/ARB treated with 25 mg spironolactone for 2 months showed significant reduction in proteinuria (by 32% from baseline level of 3718 mg/24 h) and blood pressure (6/4 mmHg) as compared with placebo [150]	A	[150]
	Double-blind, placebo-controlled study in 59 patients with Type 2 DM and macroalbuminuria despite long-term use of ACEi/ARB treated with 25–50 mg spironolactone for 1 year showed significant reduction in albuminuria (by 41% from baseline level of 770 mg/24 h), blood pressure (7/3 mmHg) and a leveling off of GFR decline as compared with placebo [151]	A	[151]
Future clinical studies	Randomized, double-blind, placebo-controlled trial to study the effect of adding spironolactone on albumin:creatinine ratio, urine TGF- β and lipids in Type 1 and 2 DM patients with macroalbuminuria while on ACEi (NCT00381134) [222]		[222]

Quality A: Prospective, randomized, controlled trial; Quality B: Intervention trial, but not randomized or blinded; Quality C: Observational data.

ACEi: ACE inhibitor; AGE: Advanced glycation end products; ALTITUDE: Aliskiren Trial in Type 2 Diabetes Using Cardiovascular and Renal Disease Endpoints;

ARB: Angiotensin receptor blocker; CKD: Chronic kidney disease; CTGF: Connective tissue growth factor; DM: Diabetes mellitus; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease;

FGS: Focal segmental glomerulosclerosis; GFR: Glomerular filtration rate; PKC: Protein kinase C; ROS: Reactive oxygen species; STZ: Streptozotocin.

Table 3. New therapeutic agents for diabetic chronic kidney disease (cont.).

Compound	Mechanism of action and study findings	Quality	Ref.
Deferiprone	Efficiently binds catalytic iron within the kidney and removes it from participation in the generation of free oxygen radicals by chelation and urinary excretion		
Animal studies	Rats (spontaneous glomerular sclerosis model) fed on low-iron diet developed reduced levels of proteinuria Rats with remnant kidney (model for progressive renal disease) treated with deferoxamine had reduced iron accumulation in proximal tubules and tubular damage	[152] [153]	
Clinical trials	Open-label proof-of-concept study in 37 patients with Type 2 DM and microalbuminuria receiving ACEI treated with 9 months of 50 mg/kg deferiprone showed significant reduction in albuminuria (196 to 25 mg/g; p = 0.008)		[154]
Future clinical studies	None		

Quality A: Prospective, randomized, controlled trial; Quality B: Intervention trial, but not randomized or blinded; Quality C: Observational data.

ACEI: ACE inhibitor; AGE: Advanced glycation end products; ALTITUDE: Aliskiren Trial in Type 2 Diabetes Using Cardiovascular and Renal Disease Endpoints;

ARB: Angiotensin receptor blocker; CKD: Chronic kidney disease; CTGF: Connective tissue growth factor; DM: Diabetes mellitus; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease;

FSGS: Focal segmental glomerulosclerosis; GFR: Glomerular filtration rate; PKC: Protein kinase C; ROS: Reactive oxygen species; STZ: Streptozotocin.

inflammatory injury within the kidney [46]. RAGE mediates intracellular signaling pathways leading to activation of p38 kinase, extracellular signal-related kinase, MAPKs, PKC, ROS and NF- κ B-mediated growth factors including TGF- β , VEGF and CTGF [47]. This process is an important mechanism to the pathogenesis and progression of diabetic CKD [48]. In the glomeruli of patients with diabetic CKD, RAGE expression is upregulated, and this increased activity correlates with the AGE accumulation [47]. The oxidative, carbonyl and nitrosative stresses in diabetes may further potentiate AGE-induced diabetic complications by facilitating further formation of AGEs and crosslinking collagen [49]. Finally, soluble RAGE is the extracellular portion of the RAGE receptor that binds to AGEs, and thus may act as a functional antagonist to the full-length RAGE [47]. The AGE inhibitors act by reduction of total AGE content or by modification of AGE and consequent deactivation, thus offering multiple opportunities for intervention in diabetic CKD. We review pyridoxamine in detail, and briefly describe the other therapies targeted at AGE (Table 2).

Properties

Pyridoxamine is the active form of vitamin B6 and was first identified as an 'Amadorin' (i.e., a post-Amadori AGE inhibitor) [50]. Pyridoxamine has been gaining popularity owing to its therapeutic effect on diabetic complications [51]. Its proposed mechanism of action includes:

- Inhibition of AGE formation by blocking the oxidative degradation of Amadori intermediates
- Scavenging of toxic carbonyl products of glucose and lipid degradation
- Trapping of ROS [52]

Pyridoxamine has also been shown to protect the cell–matrix interactions in renal glomeruli, which are weakened by the carbonyl stresses of diabetes [53]. Recently, a second-generation Amadorin called BST-4997 has been developed, which has greater post-Amadori potency than pyridoxamine, but has no dicarbonyl scavenging activity [37].

Animal studies

In STZ diabetic rats, pyridoxamine has been demonstrated to inhibit the progression of albuminuria, attenuate the rise in creatinine, improve hyperlipidemia and reduce AGEs and crosslinking of skin collagen [54]. In another study on STZ diabetic rats, pyridoxamine was more effective

than ACEI, antioxidant vitamin E and lipoic acid in protecting the renal function [55]. In Type 2 diabetic KK-A(y)/Ta mice, pyridoxamine improved albuminuria and reduced AGE accumulation and TGF- β expression in the kidney [56]. Additionally, in obese Zucker rats (animal model for Type 2 DM), pyridoxamine reduced collagen AGEs, improved hyperlipidemia, reduced albuminuria and attenuated the rise in creatinine [57]. Finally, the combination of enalapril and pyridoxamine was shown to reduce both mortality and progression of established kidney disease in the db/db mouse model (genetic model for Type 2 DM) [58].

Clinical trials

To date, two Phase II trials have been conducted to study the effect of pyridoxamine on patients with diabetic CKD; they were later merged for *post-hoc* analysis [59] in order to better compare the results with those of landmark studies using ARB therapy [13,14]. PYR-205/207 and PYR-206 were both randomized, double-blind, placebo-controlled, multicenter trials with similar designs, which recruited patients with Type 1 and 2 DM patients with macroalbuminuria (>300 mg/24 h) and serum creatinine of less than 2 mg/dl in PYR-205 and 207 and 2.0–3.5 mg/dl in PYR-206. The patients were randomized to 250 mg twice daily for 20 weeks after dose escalation or matching placebo in PYR-205/207, and 50 mg twice daily for 24 weeks or matching placebo in PYR-206. These studies were primarily designed to evaluate the safety and tolerability of pyridoxamine.

Overall, a total of 212 patients with diabetic CKD (baseline serum creatinine 1.5 mg/dl and urine albumin of 1058 mg/24 h) were randomized. Although pyridoxamine was generally well tolerated and the differences between the adverse events in the pyridoxamine and placebo groups were generally not statistically significant, an imbalance in the number of serious adverse events was evident in PYR-205/207. The most frequently reported serious adverse events were cardiovascular events or infections, and hence not considered by the investigators to be related to the study drug. However, a total of four deaths occurred in the pyridoxamine groups, with no deaths in the placebo groups. Nevertheless, only one of the deaths could potentially be attributable to the study drug.

In the merged dataset, pyridoxamine significantly reduced the change in serum creatinine from baseline (-48%; $p < 0.028$). Furthermore, in a subset analysis of a population similar to those

enrolled into the Irbesartan in Diabetic Nephropathy Trial [13] and the RENAAL [14] study, the serum creatinine change from baseline was 0.06 mg/dl for pyridoxamine versus 0.29 mg/dl for placebo ($p = 0.007$). No differences in urinary albumin excretion were seen in any patient population. Urinary TGF- β 1 also tended to decrease in patients receiving pyridoxamine ($p = 0.049$).

Future clinical trial

A Phase II clinical trial of K-163 (oral pyridoxamine) in inhibiting AGE production in patients with diabetic CKD is planned by KOWA in Tokyo, Japan.

Expert commentary

Renal damage due to AGEs has been well documented, and presents several opportunities for AGE inhibition at the formation or receptor activation stage. The current AGE inhibitors in clinical development have different sites of inhibition, safety, tolerability and efficacy. As agents of this class progress through clinical trials, more will be discovered about the use of AGE inhibitors in diabetic CKD and other diabetic microvascular complications, including retinopathy, neuropathy, poor wound healing and gastroparesis. Since the cellular mechanisms by which AGE/RAGE mediate injury, practical trials that evaluate broad cardiovascular, renal and infection/sepsis composite end points will be warranted [60].

Protein kinase C inhibitors

Background

Protein kinase C is a family of serine–threonine protein kinases consisting of 12 isoforms that are involved in intracellular signaling [61]. PKC regulates many vascular functions, including permeability, contractility (vasodilator release), endothelial activation, growth factor signaling (extracellular matrix and cell growth) and adhesion of monocytes and leukocytes. These are processes that are known to be deranged in patients with DM [61]. Hyperglycemia increases diacylglycerol production and activates PKC in the endothelial cells, smooth muscle cells and renal mesangial cells [62]. Other mechanisms for increased PKC activity in the diabetic milieu include ROS [63] and AGEs [64].

The upregulated PKC activity is proposed to cause diabetic CKD by mediating glomerular hyperfiltration. The likely molecular mechanisms include enhancement of angiotensin II vasoconstrictor action [65], increased nitric oxide

production [66] and VEGF expression [67]. PKC has been shown to cause accumulation of extracellular matrix by increasing the expression of TGF- β [68]. PKC activation can also increase vascular permeability to albumin [69]. Thus, inhibiting activated PKC isoforms with specific drugs in DM may ameliorate microvascular damage. Although PKC- α and - β activation have been shown to be important in diabetic CKD [70], most animal and clinical studies have evaluated the selective PKC- β inhibitor ruboxistaurin.

Properties

Ruboxistaurin mesylate monohydrate (RBX) is a macrocyclic bisindolylmaleimide compound (LY333531) and a selective inhibitor of PKC- β I and - β II isoforms [70]. RBX binds to the active site of the PKC- β molecule and interferes with the binding of ATP, thus inhibiting the ability of PKC- β to phosphorylate substrates [70]. RBX is available in an oral formulation, and has been shown to be well tolerated at 32 mg/day in trials of patients with diabetic retinopathy with an adverse-effect profile similar to placebo [71].

Animal studies

In STZ diabetic rats, RBX prevented TGF- β overexpression and extracellular fibronectin and α 1(IV) collagen in the glomeruli [72]. In another study, Type 2 DM obese db/db mice treated with oral RBX reduced albuminuria and prevented mesangial expansion [73]. A recent study showed that Ren-2 rats (transgenic model of hypertension made diabetic with STZ) treated with RBX had a reduction in albuminuria, TGF- β expression and glomerular injury, despite ongoing hypertension and hyperglycemia [74]. These studies used RBX prior to the development of diabetic CKD, and thus the effect of RBX on established CKD is unknown.

Clinical trials

In a pilot trial, 123 patients with Type 2 diabetes and persistent macroalbuminuria (ACR: 200–2000 mg/g) despite renin–angiotensin system inhibitors were randomized to treatment with either RBX 32 mg/day for 1 year or placebo [75]. Patients treated with RBX had a significant reduction in albuminuria (by 24%; baseline: 724 mg/g) and stable renal filtration function (baseline eGFR: 71 ml/min/1.73 m²). An estimation of urinary TGF- β in 107 patients from this study revealed a nonsignificant change (+19%) in urinary TGF- β in the RBX group, while a significant increase (+43%) was observed

in the placebo group [76]. Analysis of renal outcomes in a long-term (approximately 3-year) study of RBX for diabetic retinopathy showed no significant difference in doubling of serum creatinine or advanced CKD between the RBX- and placebo-treated groups [77]. These trials were limited by the size of the study sample, and thus larger prospective clinical trials are needed to assess the true benefit of RBX on clinical outcomes in diabetic CKD.

Future clinical trials

A Phase III clinical trial (NCT00297401) is currently recruiting patients with Type 1 DM and microalbuminuria (urine ACR >20 mg/mmol) to study the effect of RBX on albumin excretion [204].

Expert commentary

Inhibition of the overactive PKC in diabetes remains yet another promising therapeutic option. Since different PKC isoforms mediate diabetes-induced microvascular damage in different organs, clearly defining the specific PKC renal pathways will be a key milestone. The results of the Phase III clinical trial of RBX are eagerly awaited to confirm the positive results of the Phase II studies. If safety and efficacy are demonstrated, large long-term clinical trials will be needed to establish the efficacy of PKC inhibitors in the treatment of diabetic CKD, with broad morbidity and mortality end points.

Other therapeutic agents

Numerous other drugs are being tested for their safety and efficacy in preventing the progression of diabetic CKD (Table 3). Renoprotection by the action of thiazolidinediones on TGF- β looks promising, but may have to be used with caution in light of a recent concern for increased cardiovascular risk with rosiglitazone in Type 2 diabetics [78]. Few small randomized trials have shown a beneficial effect of adding spironolactone to recommended antihypertensive treatment with further reduction in proteinuria; this needs to be studied in larger trials. Preliminary studies of PPAR- α agonists (fenofibrate), endothelin receptor antagonists and aldose reductase inhibitors have shown mild benefit, and must be evaluated in larger trials for safety and efficacy.

One of the most promising approaches in diabetic CKD involves the recognition of catalytic iron in the participation of oxidative stress and the *in situ* creation of ROS within the kidney. Deferiprone efficiently binds catalytic iron within the kidney and removes it from participation in

the generation of free oxygen radicals by chelation and urinary excretion. Trials are planned with this agent for both acute and chronic forms of kidney disease [79]. Together, the extensive list of agents in Table 3 attests to the complex pathways for renal damage in diabetes and the multiple targets possible to retard the progression of diabetic CKD.

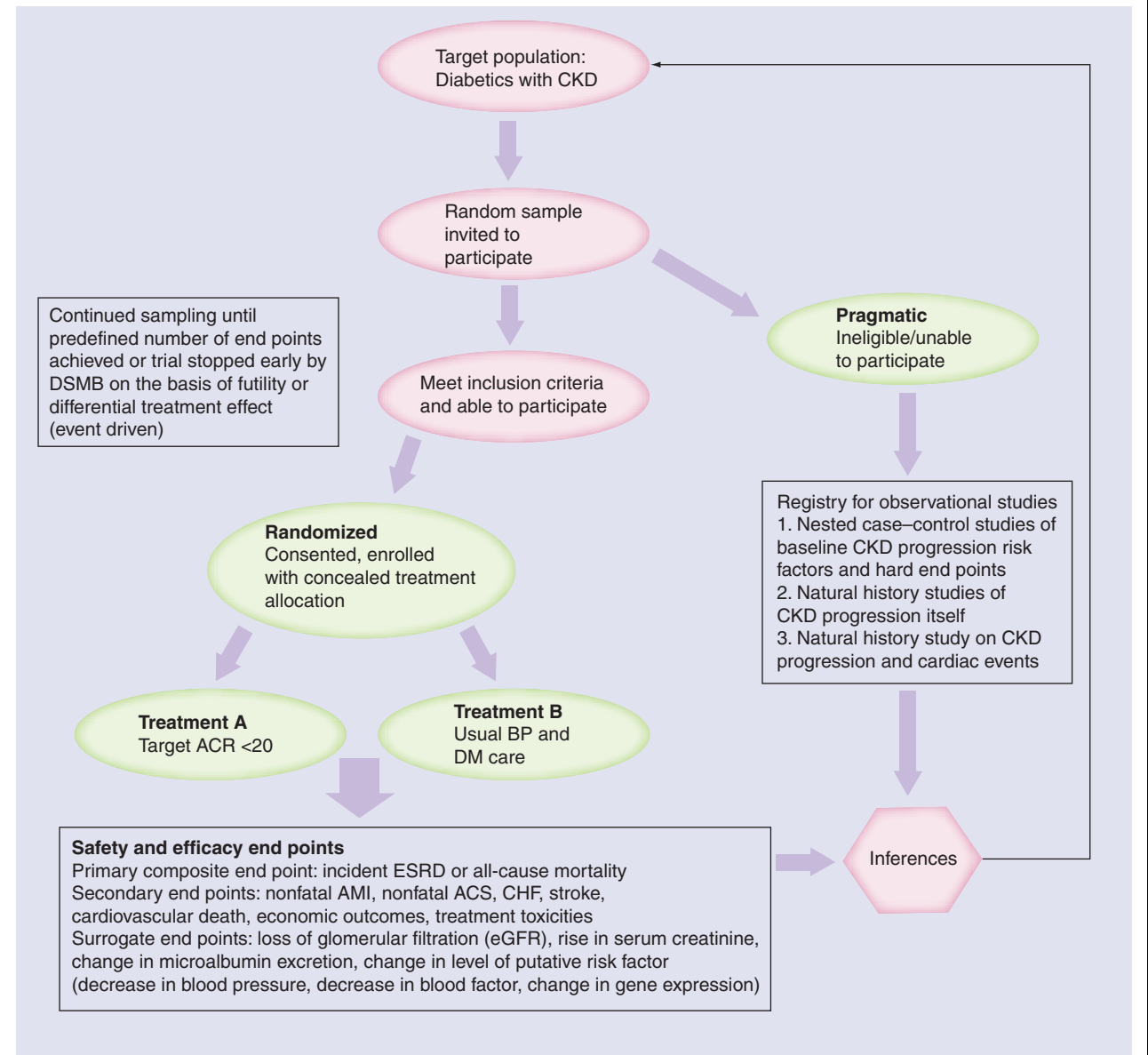
Conclusion

Extensive research during recent years has identified several new pathways in the pathogenesis of hyperglycemia and hypertension-mediated CKD. Accordingly, many potential targets to prevent the development or retard the progression to ESRD are currently under evaluation. GAGs, AGE inhibitors and PKC inhibitors are being studied for safety and efficacy in diabetic CKD. These new therapeutic agents may reduce the risk for ESRD and prove to be a valuable addition to the treatment armamentarium for this common clinical problem.

Future perspective

The micro- and macro-vascular complications of diabetes are a serious cause for morbidity and mortality. Exploring the molecular mechanisms by which diabetes causes vascular complications is complex, yet exciting. New therapeutic agents that show promising results in animal models of diabetes must be studied for safety and efficacy in humans. The renal benefits that need to be studied include reduction in urine albumin excretion, preventing the progression of microalbuminuria to macroalbuminuria, slowing the decline in glomerular filtration rate and reducing the incidence of ESRD. Studies should assess if these effects are independent of blood pressure or glycemic control and supplement current therapy. New blood and urinary biomarkers for diabetic CKD are needed to further aid in the development of novel therapeutic compounds. While establishing the efficacy for treatment of diabetic CKD, the use of these new agents in improving other vascular complications – diabetic retinopathy, neuropathy and cardiovascular disease – should also be studied. An ideal clinical trial of a novel therapy for diabetic CKD would have combined renal and cardiovascular outcomes with embedded practical measures of cost and clinical effectiveness, as shown in Figure 2. Finally, the prevention of diabetic CKD would be the ultimate goal, with the elimination of the need for dialysis and its related comorbidities.

Figure 2. Schema for ideal, pivotal randomized trial of a novel agent in the treatment of diabetic CKD.



ACR: Albumin:creatinine ratio; ACS: Acute coronary syndromes; CKD: Chronic kidney disease; CHF: Congestive heart failure; DM: Diabetes mellitus; DSMB: Data Safety Monitoring Board; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease. Modified from [155].

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Executive summary

- Diabetic chronic kidney disease (CKD) is the leading cause of end-stage renal disease (ESRD).
- Current management strategies employ aggressive glycemic and blood pressure control combined with renin–angiotensin–aldosterone system-blocking agents to slow the worsening of kidney disease.
- Despite use of these drugs, progression to ESRD still occurs and, hence, there is the need to develop new therapeutic agents.
- The complex pathogenesis of diabetic CKD offers multiple opportunities for development of drugs targeting different steps of the pathways leading to renal damage.

Glycosaminoglycans

- Glycosaminoglycans (GAGs) have been proposed to act by restoring GAG content present in reduced amounts in the glomerular basement membrane of diabetic animal models.
- Thus, GAGs improve the function of the glomerular basement membrane as a charge-selective barrier.
- In animal models, sulodexide was shown to reduce albuminuria and TGF- β activity.
- Oral sulodexide has been shown in many Phase II clinical trials to reduce albuminuria in diabetic CKD.
- The Diabetic Nephropathy and Albuminuria Sulodexide study was a large multicenter European trial that demonstrated significant reduction in albuminuria regardless of Type 1 or Type 2 diabetes, blood pressure control or glycemic control. Sulodexide accentuated the albuminuria reduction in patients receiving angiotensin-converting enzyme inhibitors.
- Phase III and IV clinical trials have failed to show benefit in Type 2 diabetes patients.

Advanced glycation end product inhibitors

- Non-enzymatic glycosylation of proteins in the diabetic milieu leads to the formation of advanced glycation end product (AGEs).
- Receptors for AGEs are expressed on renal tubular epithelial cells, mesangial cells, podocytes and circulating inflammatory cells and mediate multiple intracellular signaling processes, leading to tissue damage.
- AGE deposition in multiple organs in diabetics is associated with oxidative stress and crosslinking of collagen.
- Inhibitors of AGEs reduce the formation, accumulation and receptor-mediated action of these pathogenic substrates.
- In animal models, pyridoxamine was shown to reduce albuminuria, TGF- β activity and AGE accumulation.
- *Post-hoc* analysis of two Phase II clinical trials of oral pyridoxamine showed favorable safety profiles and a slower rise in serum creatinine, with no change in albuminuria.
- A Phase II clinical trial in Japan is planned.

Protein kinase C inhibitors

- Protein kinase C (PKC) inhibitors suppress the upregulated activity of this intracellular kinase and attenuate renal damage.
- In animal models, ruboxistaurin has been shown to reduce albuminuria, TGF- β activity and extracellular matrix accumulation.
- Oral ruboxistaurin has shown good tolerability in studies for diabetic retinopathy.
- Phase II clinical trials of ruboxistaurin demonstrated mixed results, with reduction in albuminuria and preserved renal filtration function but no effect on doubling of serum creatinine in 3 years.
- A Phase III clinical trial of ruboxistaurin is planned.

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