Novel therapies of diabetic nephropathy
Basil O. Burney, Rigas G. Kalaitzidis and George L. Bakris

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Sulodexide

Sulodexide is an oral formulation of highly purified mixture of glycosaminoglycans. It is composed of 80% fast-moving heparin sulphate and 20% dermatan sulphate and is the most extensively investigated glycosaminoglycans for diabetic patient [9–11]. It bears strong chemical similarity to heparin but does not have anticoagulation properties when given orally. Sulodexide has emerged as potential treatment of diabetic nephropathy as multiple studies demonstrate reductions in urinary albumin excretion with glycosaminoglycan therapy [12–14].

The precise physiology of the sulodexide-mediated nephroprotection in diabetic nephropathy is not clear, but several mechanisms have been described. In-vitro sulodexide has been shown to inhibit heparinase HPR-1 activity [15]. HPR-1, which is upregulated in high-glucose conditions, is responsible for decreasing the proteoglycan content of the glomerular basement membrane (GBM) by degrading heparin sulphate proteoglycans. As sulodexide is a mixture of glycosaminoglycans, it helps in restoring the glomerular glycoproteins present in GBM and mesangium. Another mechanism involves restoring the anionic heparin sulphate charge on the GBM by sulodexide and its related compounds. Finally, it suppresses high-glucose induced overexpression of TGF-β1 that is responsible for enhanced expression of mesangial matrix and collagens [16].

The efficacy of sulodexide in diabetes was evaluated in DiNAS study [17]. DiNAS was a randomized, double blind and placebo controlled trial involving 223 patients with microalbuminuria and macroalbuminuria who had type 1 or type 2 diabetes and stable blood pressure. Patients were randomized to sulodexide 50, 100 and 200 mg daily and placebo for 4 months with a 4-month observation period after drug discontinuation. After 4 months of therapy, albuminuria decreased by 30, 49 and 74%, respectively, compared with the placebo group. Four months after drug discontinuation, albuminuria remained 69% lower in those randomized to 200 mg of sulodexide compared with the placebo group. There were no statistical differences in albuminuria reduction from placebo in the other groups. This sustained response suggested that some anatomical or structural change had occurred with sulodexide. Sulodexide was well tolerated and no major side-effects were seen in this study.

A recent pilot study included 149 patients with type 2 diabetes and microalbuminuria, defined as an albumin/creatinine ratio (ACR) between 20 and 300 mg/g creatinine. These patients were randomized to 200 and 400 mg of sulodexide versus placebo. The primary endpoint at 6 months was a 50% reduction in ACR or return to normoalbuminuria. This was achieved in 33.3, 18.4 and 15.4%, respectively [18**]. Given the positive finding from these smaller studies, the sulodexide microalbuminuria trial (SUN-Micro-Trial) [19] was designed that evaluated the effects of sulodexide on diabetic nephropathy. It was a randomized, double blind, placebo controlled trial involving 1000 patients with diabetes and persistent microalbuminuria who were already receiving maximal RAAS blocking therapy. The primary endpoint was reduction in the urine albumin. However, this trial failed to achieve the primary endpoint. With the failure of this trial to demonstrate reduction of albuminuria in diabetic nephropathy, another already planned phase 4 trial, the SUN-Macro-Trial, was cancelled. Thus, a promising therapy tested early in its development on change in a marker of nephropathy progression, that is microalbuminuria failed to perform in an adequately powered study.

Ruboxistaurin

Overexpression of PKC is another key metabolic pathway involved in the pathogenesis of the diabetic nephropathy. PKC is a family of at least 12 isoforms that play an important role in signal transduction. PKC is activated in response to diacylglycerol, which is increased in hyperglycaemia [4**]. Activated PKC causes kidney damage through number of mechanisms including generation of oxidants through activation of NADPH oxidase leading to oxidative stress [20] and signalling TGF-β to induce extracellular matrix production [21]. Ruboxistaurin (RTX) is an oral PKC-β inhibitor and, in animal studies, has been shown to normalize glomerular hyperfiltration, reduce extracellular matrix protein production and TGF-β1 and ultimately decrease albuminuria [22].

In a recent pilot study of 123 patients with diabetic nephropathy and macroalbuminuria, patients were randomized to either 32 mg daily of RTX or placebo for 1 year [23]. Patients in both arms were continued on angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) during this trial. The primary endpoint was reduction in urinary albumin excretion. After 1 year, patients treated with RTX experienced 24% reduction in albuminuria as compared with those treated with placebo. This reduction in albuminuria appeared as early as 1 month following treatment initiation. In RTX group, kidney function assessed by estimated glomerular filtration rate (eGFR) was stable, whereas in placebo group, there was a decline in eGFR. However, this study had some limitations. It was underpowered to detect any significant differences in albumin-creatinine ratio and eGFR. Another limitation of this study was its small size study and limited duration of follow-up that further limited conclusions about safety of RTX.

RTX has also been shown to reduce visual loss by 40% in patients with moderate to severe diabetic retinopathy.
after 3 years compared with placebo patients [24,25]. This was noted in PKC Diabetic Retinopathy study 2 (PKC-DRS2). However, in the same trial, investigators reported adverse event of ‘diabetic nephropathy’ more frequently in patients receiving RBX than those receiving placebo \( n = 7 \) (2%) versus \( n = 0; P = 0.015 \). Recently, a long-term, extensive and collective evaluation of three diabetic retinopathy trials compared the effects of RTX on kidney function [26**]. The results demonstrate that there was no difference in the frequency of elevated urine albumin excretion, eGFR or kidney outcomes in the two arms of the study. A recent drug safety analysis [27] of patients from different RBX studies failed to confirm an increased rate of diabetic nephropathy previously reported by investigators in PKC-DRS2 study. With these mixed results from different small clinical trials involving RTX, a large-scale randomized prospective trial is required to study the benefits and safety of RBX in patients with diabetic nephropathy. Unfortunately, due to the results of the retinopathy trial, the Food and Drug Administration required further testing and did not give approval for retinopathy, consequently, the company failed to develop the product further.

### Pyridoxamine

There is increasing evidence to support the concept that advanced glycation end products (AGEs) are involved in the pathogenesis of diabetic nephropathy and other diabetes complications [28]. In diabetes, AGEs are formed by the modification of protein amino groups by glucose. The formation of AGEs increases not only by increase in oxidative substrate such as glucose in diabetes, but also by increase in reactive carbonyl species and reactive oxygen species. This important role of AGEs in the pathophysiology of diabetic kidney disease resulted in development of different compounds including pimegadine, tested in a clinical trial and, although efficacious, had safety issues [29] and more recently, pyridoxamine, an active inhibitor of AGEs [30]. The precise mode of pyridoxamine’s action is still not clear, but it likely acts through three different mechanisms: inhibition of glycated proteins (Amadori products) breakdown, reduction of toxic effects of reactive oxygen species and scavenging of reactive carbonyl compounds [30].

Pyridoxamine has been studied extensively in rat models. In two previous rat models of type 1 and type 2 diabetes, pyridoxamine preserved kidney function [30–32]. A phase 2 trial PYR-206 studied the safety and efficacy of pyridoxamine. A total of 128 patients with diabetic nephropathy received either 50 mg of pyridoxamine or placebo for 6 months [33*]. Another similar phase 2 trial PYR-205/207 included 84 patients with either type 1 or type 2 diabetes [33**]. In these trials, patients were randomized to receive either pyridoxamine 250 mg or placebo in addition to standard of care therapy for diabetic nephropathy. As both trials were similar, merged data sets were published [33**]. The data revealed a reduction of baseline serum creatinine of 48% in pyridoxamine group as compared with placebo group. Pyridoxamine group also decreased urinary excretion of TGF-β as compared with baseline. No change in urinary albumin excretion (UAE) was noted in either treatment or placebo groups. Moreover, there was no change in UAE in either group from baseline.

The results of this integrated study suggested positive effects of pyridoxamine on slowing the progression of the diabetic kidney disease as witnessed by improvement in serum creatinine levels from baseline in the treatment group. These phase 2 trials demonstrate that pyridoxamine is well tolerated in patients and has a positive impact on the preservation of the renal function in patients with diabetic nephropathy. One must be cautious, however, in interpreting these small studies and a large trial such as that with sulodexide needs to be performed to assess the true benefit, if any.

### Aliskiren

Aliskiren is a highly potent and orally effective renin inhibitor. It is responsible for inhibiting renin and, thus, decreasing angiotensin I and angiotensin II levels as well as aldosterone levels. Aliskiren also significantly reduces the expression of TGF-β, a protein which in conjunction with angiotensin 2 plays an important role in progression of renal fibrosis in diabetic nephropathy [4**]. The antihypertensive effects of aliskiren are clear and it was approved for treatment for hypertension in 2007 [34]. Moreover, it has proven additive antihypertensive effects when used in combination with other blood pressure lowering agents [35*].

Aliskiren decreases albuminuria, lowers blood pressure and normalizes serum creatinine in transgenic rats for human renin and angiotensinogen genes [36]. In another rat model, aliskiren in comparison with perindopril reduced blood pressure to the same extent as perindopril but attenuated tubulointerstitial fibrosis more than perindopril did, an important factor in diabetic nephropathy [37*]. It also reduced albuminuria and glomerulosclerosis similar to the levels achieved by perindopril [37*]. Feldman et al. [38] noted similar findings on blood pressure and albuminuria lowering as well as suppression of TGF-β in a different rat model.

A small study of 15 patients with diabetes received 300 mg of aliskiren [39]. In this study, urinary albumin–creatinine ratio (UACR) was reduced by 17% in 2–4 days and 44% at 28 days. Blood pressure also fell appropriately. This interesting observation was recently testing properly when dual
blockade of the RAAS system was studied in the Aliskiren in the Evaluation of proteinuria in Diabetes (AVOID) trial [40**]. This was a randomized double-blind study involving 599 patients. Participants enrolled in this study entered into a 3-month open-label period where any previously administered drug that interfered with RAAS was discontinued, except beta-blockers. Treatment was initiated with 100 mg of losartan in all participants and then patients were randomly assigned to either aliskiren (150 mg for 3 months titrated to 300 mg for next 3 months) or placebo for total of 6 months. The primary outcome was reduction in UACR. A 20% reduction in UACR was observed in the aliskiren group when compared with placebo. About twice as many patients who received aliskiren had a more than 50% reduction in albuminuria compared with those receiving placebo.

The results of these animal and human studies are very promising, and the results of a large outcome trial in diabetic nephropathy, ALTTITUDE [41] (Aliskiren Trial in Type 2 Diabetes using Cardiovascular and Renal Disease Endpoints) is awaited. This large-scale randomized placebo controlled trial will compare aliskiren at a dose of 300 mg daily to placebo in presence of conventional therapy in patients with type 2 diabetes who are at high risk for cardiovascular and renal mortality and morbidity. The primary endpoint of this study would be doubling of serum creatinine, onset of end-stage renal disease (ESRD), cardiovascular related death, myocardial infarction and stroke.

Conclusion
Current established treatments for diabetic nephropathy all involve blood pressure reduction on a base of RAAS-blocking therapy that includes ACEIs and ARBs. The last decade has seen new and interesting agents tested in the context of different pathophysiologic mechanisms of diabetic nephropathy. These novel treatments were targeted to specific mechanisms in the pathogenesis of diabetic kidney disease. Out of the newer drugs, there is solid evidence to support renin inhibition, although outcome data are pending. All other innovations have failed to demonstrate clear benefit or in the case of pyridoxamine need more studies. On the basis of the available trial data, an updated paradigm for approaching a patient with diabetic nephropathy was recently published by the American Society of Hypertension [42**].

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest
Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 167–168).

3 Comprehensive review of antihypertensive agents on changes in proteinuria in the context of kidney disease progression.
6 A review of cytokine and hormonal mechanisms involved in the development of diabetic nephropathy.
10 A review that integrates the immune, inflammatory and hormonal factors that contribute to development to nephropathy.


A review of studies with this PKC-β inhibitor in diabetes.


