Pyridoxamine, Advanced Glycation Inhibition, and Diabetic Nephropathy

Joline L. T. Chen and Jean Francis

The Renal Section, Boston University School of Medicine, Boston, Massachusetts

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Diabetic nephropathy remains the most common cause of ESRD, accounting for more than 40% of patients treated with dialysis. The standard therapy for diabetic nephropathy consists of early detection, aggressive glycemic control, and treatment of hypertension and proteinuria using renin-angiotensin system (RAS) blockade. Both angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are effective in slowing the progression of diabetic nephropathy.1,2 Despite the standard of care, many patients continue to progress, and the prevalence of patients with ESRD secondary to diabetic nephropathy continues to rise.3 More effective agents are sorely needed.

The molecular pathogenesis of diabetic nephropathy is not fully understood, but both hemodynamic and metabolic mechanisms are likely involved. Tackling the hemodynamic factors with RAS blockade has proven beneficial.1,4 Many therapeutic agents that may be able to interfere with metabolic pathways are currently under investigation, and a few may be promising. For example, reduced insulin receptor signaling on the podocytes due to insulin resistance may accelerate diabetic kidney changes, and insulin sensitizers such as thiazolidinediones reduce albuminuria in clinical studies.5,6 Albumin-induced oxidation products activate stress genes and tubular apoptosis in diabetic nephropathy.7,8 Chronic inflammation and increased oxidative stress are also frequently associated with endothelial and mesangial cell injury, mesangial space expansion, and diabetic glomerulosclerosis, and the antioxidant and inflammation modulator, bardoxolone methyl, slows the progression of disease in patients with advanced diabetic nephropathy.9 The antifibrotic agent pirfenidone also decreases the rate of decline in estimated GFR among patients with diabetic kidney disease.10

Studies using other agents, however, have been disappointing. While endothelin expression is increased in diabetic nephropathy and has potent vasoconstrictive, proinflammatory, and profibrotic effects, its blockade using avosentan does not deter diabetic nephropathy and may increase risk of heart failure and edema.11 Also, sulodexide, a glycosaminoglycan that can alter the glomerular capillary barrier and reduce albuminuria in animal models, failed to decrease albuminuria in patients with type 2 diabetes.12,13

Persistent hyperglycemia modifies lipids and proteins by nonenzymatic covalent binding of sugar residues through a series of complex biochemical reactions, leading to the formation of advanced glycation end products (AGEs).14 High levels of AGEs are present in diabetic patients, and experiments in animal models suggest that AGEs induce direct injury to the mesangial cells and podocytes as they upregulate gene expression of collagen and TGFβ1 in diabetic glomerular lesions.15 AGEs mediate their actions by receptor-dependent or -independent mechanisms. The receptors for AGEs are expressed on podocytes, and the inhibition of their activity reduces the expression of TGFβ, mesangial expansion, and basement membrane thickening.16 Therefore, inhibition of AGE formation seems to be an attractive therapeutic option that may alter the pathogenesis and delay the progression of diabetic kidney disease. Agents that have been found to affect tissue and/or circulating AGE levels include aspirin, thiamine, thiazolidinediones, carnosines, ACEIs, ARBs, and pyridoxamine.17 The fact that many of these agents also have direct hemodynamic effects may be confounding.

Pyridoxamine (Pyridorin; NephroGenex, Inc.) inhibits the formation of AGEs from glycated proteins and by trapping pathogenic reactive carbonyl compounds, the intermediates in the formation of AGEs.18 It delays the development of diabetic nephropathy and reduces albuminuria in animal models of both type 1 and type 2 diabetic nephropathy.19,20 Two phase II clinical studies were conducted in patients with mild to moderate type 1 and type 2 diabetic nephropathy, and the merged results were analyzed post hoc by Williams et al.21 In this study, the data from 65 patients assigned to 50 mg of pyridoxamine twice daily compared with 63 patients receiving placebo for 24 weeks and that of 57 patients taking 250 mg of pyridoxamine twice daily compared with 27 patients taking placebo for 20 weeks were combined for evaluation of safety and efficacy. In separate analyses, pyridoxamine had some or little effect on change in renal function compared with baseline. However, after merging the data, pyridoxamine appeared to have a significant effect in reducing the slope of creatinine change from baseline compared with placebo (percent serum creatinine changed = -48%, P=0.03), and the effect was even more impressive among the subgroup with type 2 diabetes and baseline serum creatinine >1.3 mg/dl. Although pyridoxamine did not affect urine albumin excretion, it significantly reduced plasma AGE level compared with the placebo group. Whereas the result here was encouraging, the posteriori and subgroup analyses might be associated with unintended biases. Notably, the authors documented a higher incidence of serious adverse events such as cardiovascular events or infections among the higher pyridoxamine dose group, but the events were thought to be unrelated to the medication.
In this issue of JASN, Lewis et al. and the Collaborative Study Group report the data from their double-blind randomized controlled trial using pyridoxamine to treat patients with type 2 diabetes and overt proteinuria. A total of 317 patients were randomized to placebo or 150 mg twice daily or 300 mg twice daily of pyridoxamine for 12 months. Patients were required to have serum creatinine between 1.3 and 3.5 mg/dl and a 24-hour urine protein to creatinine ratio $\geq$1200 mg/g. All patients were already receiving the maximum recommended doses of ACEIs or ARBs and BP medications. The primary end point was defined as therapeutic success, looking for a significant difference in mean change of serum creatinine among the groups. Predetermined subgroup analyses of patients with serum creatinine $\leq$2 or $>$2 mg/dl and in tertiles were also planned.

After 1 year of follow-up, the authors described no significant change in renal function within either group and no significant differences comparing the treatment groups with the placebo group. However, further analysis showed a treatment interaction with baseline serum creatinine as a continuous variable. Subgroups analysis also suggested a trend toward treatment effect among patients with lower creatinine ranges. Further analyses using cystatin-C, estimated GFR, or proteinuria did not show any significant change or differences. Although the authors did not attribute increased adverse events or mortality to pyridoxamine, more deaths were reported among the treatment groups.

The strength of this study is its randomized, prospective design. The groups were well balanced, and the authors clearly maximized the use of RAS blockade and BP control. The compliance rate appears to be excellent throughout the follow-up period. Their findings are in contradiction to those of the study by Williams et al., although patient characteristics were similar. One possibility for the failure to show benefit is this study calculated the necessary sample size based on data from the previously mentioned pyridoxamine study and the Irbesartan Diabetic Nephropathy Trial (IDNT). Although the patient populations in IDNT also had macroalbuminuria and established renal disease, the goal was to evaluate the effect of ARBs; therefore, not all of their patients had the benefit of RAS blockade. In contrast to the IDNT study patients, the pyridoxamine study population was already on maximum angiotensin inhibition and possibly would already have a slower decline of GFR with treatment. To evaluate the benefit of pyridoxamine on top of the maximal RAS blockade, a larger sample size may be required.

We agree with the authors that the effect of advanced glycation inhibition may be seen before onset of significant pathologic changes. The study population with proteinuria $\geq$1200 mg/d or creatinine $>1.3$–1.5 mg/dl may represent patients with a significant degree of global sclerosis and interstitial fibrosis. Despite the caveats of subgroup analyses, the authors present results that are encouraging. It is possible that the effect of AGE production likely occurs earlier in the pathogenesis of diabetic nephropathy. Later in the process of diabetic nephropathy, hemodynamic effects of RAS blockade likely dominate. Thus, pyridoxamine should be examined earlier in the disease process with the use of harder clinical outcomes, such as pathologic change, measured GFR, or even time to ESRD if it is feasible.

Although both this study and the study by Williams et al. did not find a significant association between pyridoxamine and serious adverse events, the observation of more adverse events among the treatment group is concerning. Although no causality was established, any future studies involving pyridoxamine in the diabetic population should include special evaluation of its safety profile.

Many of the new agents for diabetic nephropathy remain promising, but unlikely curative. Although the findings in this study are largely negative, some aspects of this trial are encouraging. As diabetic nephropathy remains an important cause of ESRD, further study may be worthwhile to evaluate pyridoxamine’s effect in early diabetic patients with preserved renal function. However, special attention should be paid to appropriate sample size calculation for this population and examination of pyridoxamine’s safety.

**DISCLOSURES**

None.

**REFERENCES**


Vasculitis Is an Antiangiogenic State

Isaac E. Stillman* and S. Ananth Karumanchi†

*Department of Pathology and Renal Division, Department of Medicine, Beth Israel Deaconess Medical Center, and Harvard Medical School, Boston, Massachusetts; and †Renal Division, Department of Medicine and Department of Obstetrics and Gynecology, Beth Israel Deaconess Medical Center, and Harvard Medical School and The Howard Hughes Medical Institute, Boston, Massachusetts

It has been 30 years since the first description of antineutrophil cytoplasmic antibodies (ANCA).1 Elucidation of their role in systemic vasculitis has revolutionized our understanding of these diseases, and the resultant attention on the neutrophil has made it the central cell in the investigation of small vessel vasculitis.2 However, even given the developing consensus that ANCs are pathogenic,1–3 there is clearly much more to the story. In this issue of *JASN*, Le Roux et al.4 report that elevated circulating levels of soluble fms-like tyrosine kinase 1 (sFlt1), an endogenous antiangiogenic protein, are associated with ANCA-associated vasculitis (AAV) and may contribute to microvascular endothelial cell injury.

It has been evident for some time that ANCA titers correlate poorly (in both directions) with disease activity.7,8 Furthermore, there are many broader issues that remain unclear. For example, why are the lesions of a systemic process found only in particular regions of the vascular system? Renal pathologists have long recognized that the early glomerular lesion of ANCA disease, segmental necrosis, is primarily accompanied by neutrophil margination and accumulation. However, within a short time, mononuclear leukocytes come to predominate, a progression that may suggest a stepwise nature to the pathogenesis of these lesions. Interestingly, monocytes, a prominent cell of these later stages, also possess the ANCA autoantigens MPO and PR3, and the expression of the latter is increased in patients with AAV. Furthermore, in *vitro* data show that ANCA activate monocytes in a manner similar to the way they prime neutrophils.2,3 In recent years, the focus of investigation has widened to include the role of complement depletion, T cells, and even B cells. However, what of the endothelium, which remains the primary target of injury?

Not surprisingly, the endothelium has been primarily viewed through an ANCA lens, and thus much initial work was done on understanding neutrophil–endothelium

See related article, “Pyridoxin in Type 2 Diabetic Nephropathy,” on pages 131–136.


