Novel mechanisms and therapeutic options in diabetic nephropathy

Toshio Miyata

Center for Translational and Advanced Research, Tohoku University Graduate School of Medicine, Sendai, Japan

KEY WORDS

advanced glycation, hypoxia, megsin, oxidative stress, plasminogen activator inhibitor-1 (PAI-1)

ABSTRACT

Despite multiple therapeutic options, the incidence of diabetic nephropathy remains worrisome. Time has therefore come to undertake a new approach based on some breakthrough not only in medical biology but also in structural biology, chemistry, pharmacology and even computer science. Recent investigations have tried to translate several target molecules or factors identified by basic researches into clinical medicine, as delineated in this.

Classical factors contributing to the pathology of diabetic nephropathy, e.g., hypertension, hyperglycemia, hyperinsulinemia, and hyperlipidemia, are now amenable to treatment. Current therapies however do not fully prevent its renal complications. Recent studies, mainly performed in experimental animals, have identified newer culprits in the pathogenesis, such as hypoxia, advanced glycation, oxidative stress, and other bioactive molecules. Animal experiments highlight the fact that renoprotection is not necessarily linked to hemodymanic (blood pressure) or metabolic (glycemic and lipid controls) alterations but appears rather associated with an improved hypoxia, oxidative stress, and/or advanced glycation.

To assess the respective contribution of each of these mediators, small molecular weight compounds were designed to interfere with each factor or target molecule. It is indeed important to acquire tools to evaluate and confirm our hypotheses and to translate experimental results into clinical practice.

Plasminogen activator inhibitor-1 The disruption of the plasminogen activator inhibitor-1 (PAI-1) gene protects mice against diabetic nephropathy.[1] Mice lacking the PAI-1 gene escape obesity and insulin resistance.[2] A PAI-1 inhibitor might thus prove therapeutic not only as an anti-thrombotic agent but also in other clinical conditions, such as obesity, diabetes and possibly fibrotic diseases. Unfortunately, only few PAI-1 inhibitors have been identified so far and their clinical potential is yet to be evaluated.

Fortunately, the X-ray crystallographic structure for PAI-1 is available and its site for anti-protease activity has been identified. We therefore used a new approach, the structure based drug design, to obtain molecules able to bind this site and thus inhibit PAI-1 activity. Two novel, orally active, small molecule substances, TM5001 and 5007, were identified.[3] *In vitro*, they specifically inhibited PAI-1 activity and the formation of a PAI-1/tissue plasminogen activator (t-PA) complex, and enhanced fibrinolysis. *In vivo*, they efficiently inhibited coagulation and bleomycin-induced lung fibrosis.

Given to rats with Thy-1 nephritis, they reduced proteinuria and mesangial expansion (our unpublished observation), a benefit similar to that observed in the same model whose PAI-1 molecule had been mutated.[4] Clinical benefits of PAI-1 inhibitors in diabetic nephropathy remain to be demonstrated. If confirmed, these molecules might usefully expand our therapeutic armamentarium to prevent diabetic nephropathy.

Megsin Previously, we identified megsin, a novel serine protease inhibitor predominantly expressed in the kidney.[5] Its gene and protein expression augment in human and experimental kidney diseases, such as diabetic nephropathy.[6] Overexpression of megsin leads to mesangial expansion in mice aged 40 weeks.[7] Cross-breeding of megsin transgenic mice with Receptor of

Correspondence to:
prof. Toshio Miyata MD, PhD, Center for Translational and Advanced Research, Tohoku University Graduate School of Medicine, 2-1 Seiryo-Machi, Aoba-ku, Sendai, 980-8575, Japan, phone: +81-22-717-8157, fax: +81-22-717-8159; e-mail: t-miyata@mail.tains.tohoku.ac.jp
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Advanced Glycation End Products/inducible Nitric Oxide Synthase (RAGE/iNOS) double transgenic diabetic mice eventually produced a severe diabetic nephropathy similar to that observed in man.[8] We recently discovered that megsin expression increases under hyperglycemia and contributes to matrix accumulation in diabetic nephropathy by inhibiting plasmin and matrix metalloproteinases.[9]

We utilized information on the megsin protein structure to undertake the virtual screening of megsin inhibitor, as described above. Its effectiveness should be tested in animal studies.

Hypoxia Diabetic glomerular damage decreases the number of peritubular capillaries and thus oxygen diffusion to tubulointerstitial cells, leading to tubular dysfunction and fibrosis.[10] Chronic hypoxia has indeed been documented in the diabetic kidney.[11,12]

Defence against hypoxia hinges upon the hypoxia-inducible factor (HIF).[13] Its activation induces a broad range of genes (e.g., erythropoietin, vascular endothelial growth factor [VEGF], heme oxygenase type 1 [HO-1], glucose transporter [GLUT]), which eventually protect hypoxic tissues. Oxygen levels determine its stability through its hydroxylation by prolyl hydroxylase (PHD).

HIF degradation by PHD is inhibited by cobalt which substitutes for iron, an essential element for PHD activity. The role of HIF in diabetic nephropathy was thus evaluated by the provision of cobalt for 20 weeks to hypertensive, type 2 diabetic spontaneous hypertensive rats (SHR)/NDmcr-cp rats.[14] Although hypertension and metabolic abnormalities remained unchanged[15], cobalt reduced proteinuria as well as histological kidney injury. Expressions of HIF-regulated genes, including erythropoietin, VEFG, and HO-1 increased whereas the renal expressions of transforming growth factor-β (TGF-β) and of advanced glycation were significantly reduced.

Unfortunately, cobalt is too toxic to allow its use in humans but less cumbersome non-toxic small molecular activators of HIF might prove useful. Orally available, non-toxic PHD inhibitors, able to fit within the active site of PHD where HIF binds, were therefore developed.[16] The resulting correction of chronic hypoxia might protect the diabetic kidney, independently of metabolic status and blood pressure.

The recent demonstration[17] that both erythropoietin and VEGF independently accelerate diabetic retinopathy warrants caution. PHD has 3 isoforms and, fortunately, the respective role of each PHD has been elucidated. PHD2 primarily regulates angiogenesis and erythropoiesis.[18,19] By contrast, the specific disruption of PHD1 induces hypoxia tolerance by reprogramming basal oxygen metabolism.[20] A specific PHD1 inhibitor may therefore be an interesting candidate for future therapy in diabetic nephropathy.

Advanced glycation/oxidative stress Angiotensin receptor blockers (ARB) are potent inhibitors of advanced glycation end products (AGEs), both *in vitro* and *in vivo*, able to protect the kidney.[11,21,22] Still, its hypotensive effect may be poorly tolerated in patients with a normal blood pressure. A novel ARB-derivative, R-147176, was therefore designed to inhibit markedly oxidative stress and advanced glycation, without binding to the angiotensin II type 1 receptor (AT_1R) and thus virtually no anti-hypertensive effect.[23]

The inhibition of AGE formation, the AT_1R affinity, and the pharmacokinetic characteristics of 139 newly synthesized ARB-derivatives were assayed, and R-147176 was eventually selected as it strongly inhibited advanced glycation but was 6700 times less effective than olmesartan in AT_1R binding. Despite a minimal effect on blood pressure, it provided significant renoprotection in SHR/NDmcr-cp as well as in Zucker diabetic fatty rats.[23] The renal benefits of ARB thus depend on the inhibition of AGEs and oxidative stress by their chemical structure. Not only the kidney but also the brain of experimental animals were protected by similar AGE and oxidative stress inhibitory compounds.[24,25]

CONCLUSIONS The future prevention of diabetic nephropathy and of its dramatic consequences will undoubtedly rely on a multipronged approach. In addition to the current therapies insufficient to fully prevent renal complications, novel agents able to interfere with several newer culprits should provide additional, well needed benefits. Only time will tell us if renewed approaches suffices in human.

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Nowe mechanizmy i możliwości terapeutyczne w nefropatii cukrzycowej

Toshio Miyata

Center for Translational and Advanced Research, Tohoku University Graduate School of Medicine, Sendai, Japonia

SŁOWA KLUCZOWE

hipoksja, inhibitor aktywatora plazminogenu-1 (PAI-1), megzyna, zaawansowana glikacja, stres oksydacyjny

STRESZCZENIE

Częstość występowania nefropatii cukrzycowej jest, mimo wielu opcji leczniczych, wciąż niepokojąco duża. Nadszedł czas, aby przedstawić nowe spojrzenie na tę jednostkę chorobową w oparciu o pewne przełomowe osiągnięcia nie tylko w biologii medycznej, ale także w zakresie biologii strukturalnej, chemii, farmakologii, a nawet nauk informatycznych. W niniejszym artykule przedstawiono ostatnie badania nad próbą oceny znaczenia klinicznego kilkunastu kluczowych cząsteczek i czynników zidentyfikowanych przez badaczy nauk podstawowych.

Uznane czynniki ryzyka rozwoju nefropatii cukrzycowej, tj. nadciśnienie tętnicze, hiperglikemia, hiperinsulinemia oraz hiperlipidemia, poddają się aktualnym metodom leczenia. Jednak współczesne metody terapeutyczne nie są w stanie w pełni zapobiec powikłaniom nerkowym. Ostatnie doniesienia naukowe, szczególnie badania eksperymentalne na zwierzętach, dowiodły istnienia nowych patogenetycznych czynników sprawczych takich jak hipoksja, zaawansowana glikacja, stres oksydacyjny oraz pewne cząsteczki biologicznie czynne. Wnioski z doświadczeń przeprowadzanych na zwierzętach podkreślają, że nefroprotekcja niekoniecznie związana jest ze zmianami hemodynamicznymi (ciśnienie tętnicze krwi) czy metabolicznymi (kontrola glikemii we krwi i parametrów lipidowych), ale powstaje raczej w związku z nasileniem procesów niedotlenienia tkankowego, stresu oksydacyjnego i/lub zaawansowanej glikacji.

Zaprojektowano drobnocząsteczkowe związki chemiczne oddziałujące z każdym z docelowych czynników/cząsteczek tak, aby ocenić odpowiedni wpływ każdego z tych mediatorów. Jest rzeczą naprawdę ważną, aby zdobyć narzędzia do oceny i potwierdzenia stawianych przez nas hipotez, a także do przełożenia wyników badań eksperymentalnych na praktykę kliniczną.

Adres do korespondencji:
Prof. Toshio Miyata MD, PhD, Center for Translational and Advanced Research, Tohoku University Graduate School of Medicine, 2-1 Seiryo-Machi, Aoba-ku, Sendai, 980-8575, Japonia, tel.: +81-22-717-8157, fax: +81-22-717-8159; e-mail: t-miyata@mail.tains.tohoku.ac.jp

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