also been initiated to investigate whether albuminuria and proteinuria of diabetic nephropathy may be decreased after vaptan (V1/V2 mixed) treatment. To the best of our knowledge, no results of these tests have been published yet.

A frequently asked question is whether V2 vaptans increase bleeding, since DDAVP is infused to decrease bleeding in patients with von Willebrandt’s disease. Increased bleeding has not been reported in the studies conducted so far. Conivaptan, a combined V1/V2 receptor antagonist is contraindicated in liver cirrhosis; it is expected that blockade of the V1 receptor would increase flow through esophageal varices and hence might cause variceal bleeding. Recently, one laboratory has proposed that sodium sequestration into soft tissues plays a role in the regulation of extracellular sodium [13]. At this time there is no evidence that such a process has a role in hyponatraemia or in the effects of vaptans.

In summary, stay tuned! More (interesting) information about the vaptans is about to emerge in the near future.

Conflict of interest statement. Dr. Gross has been an investigator in studies involving tolvaptan, conivaptan, satavaptan and lixivaptan. The renal division received compensation for that work.

References


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Translation of basic science into clinical medicine: novel targets for diabetic nephropathy

Toshio Miyata and Charles van Ypersele de Strihou

Center for Translational and Advanced Research, Tohoku University Graduate School of Medicine, Sendai, Japan, and Service de Nephrologie, Universite Catholique de Louvain, Brussels, Belgium

Keywords: hypertension; hypoxia; metabolic syndrome; oxidative stress; PAI-1

Despite major therapeutic advances, the incidence of diabetic nephropathy remains worrisome. Classical factors contributing to its pathology (e.g. hypertension, hyperglycaemia, hyperinsulinaemia, and hyperlipidaemia) are now amenable to treatment, but current therapies do not fully prevent renal complications. The experimental study of animals has recently incriminated newer culprits, such as hypoxia, oxidative stress, advanced glycation, etc. and identified several target molecules. These progresses remain to be translated into clinical medicine.

Correspondence and offprint requests to: Toshio Miyata, Center for Translational and Advanced Research, Tohoku University Graduate School of Medicine, 2-1 Seiryo-Machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan. Tel: +81-22-717-8157; E-mail: t-miyata@mail.tains.tohoku.ac.jp

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Lessons of animal experiments for current therapies in humans

SHR/NDmcr-cp is a hypertensive rat strain derived from the spontaneously hypertensive rat (SHR). As a result of an additional mutation of the leptin receptor, it develops obesity, hyperglycaemia, hyperinsulinaemia and hyperlipidaemia, all of which characterize human type 2 diabetes mellitus [1,2]. As anticipated, significant renal damage ensues. We utilized this model to evaluate several current therapeutic approaches.

Dietary correction of obesity

Restriction of the caloric intake of SHR/NDmcr-cp by 30% for 20 weeks corrected both obesity and hyperlipidaemia but failed to change blood pressure, hyperglycaemia, and hyperinsulinaemia [2]. Nevertheless, proteinuria and histological abnormalities of the kidney were prevented. Renoprotection was thus achieved by correction of obesity despite concurrent hypertension and hyperglycaemia. Of note, renal damage correlated with body weight and with the renal content of advanced glycation end-products (AGEs) and of oxidative stress.

Normalization of blood pressure

The effect of blood pressure lowering on the kidney was assessed with several types of drugs with the hope to unravel their renoprotective mechanisms [1,3]. An angiotensin receptor blocker (ARB), a calcium channel blocker (CCB) or a beta blocker, given for 20 weeks to SHR/NDmcr-cp, were compared. Although all normalized systolic blood pressure to the same extent, only the ARB successfully decreased proteinuria. This finding fits with the clinical experience that the renin–angiotensin system (RAS) inhibitors offer a better renoprotection. Renal benefits can thus be dissociated from changes in systemic blood pressure and in metabolic abnormalities.

Impressively, ARB, but neither CCB nor beta blocker, markedly reduced the renal AGE content despite the unaltered, concomitant metabolic syndrome including hyperglycaemia [3]. ARB also corrected oxidative stress and hypoxia [3].

Control of hyperglycaemia/hyperinsulinaemia

In addition to the critical role of hyperglycaemia [4], recent studies have incriminated insulin resistance (IR) or hyperinsulinaemia in the genesis of diabetic renal injury [5]. Pioglitazone, a thiazolidinedione, lowers insulin resistance and therefore blood glucose. In order to delineate the role of insulin resistance in diabetic nephropathy, SHR/NDmcr-cp rats received for 20 weeks either pioglitazone or insulin [6]. Neither treatment modified hypertension. Pioglitazone, in contrast with insulin, decreased significantly plasma insulin levels. Despite a poorer glycaemic control, renoprotection was markedly better with pioglitazone than with insulin treatment. Of interest, both treatments reduced the renal accumulation of AGEs and markers of oxidative stress but only pioglitazone reduced renal expression of TGF-beta. The fact that insulin treatment prevents advanced glycation and oxidative stress without renoprotection reflects probably the persistence of hyperinsulinaemia and its attendant overproduction of TGF-beta [6]. Hyperinsulinaemia and the attendant increase of TGF-beta expression might therefore prove useful therapeutic targets, independently of glycaemic control, a conclusion supported by clinical evidence that pioglitazone enhances renoprotection in obese, diabetic patients with nephropathy [7,8].

Novel therapeutic targets

We should exert caution in comparing renal lesions in animals and humans. However, these experiments highlight the fact that renoprotection is not necessarily linked to blood pressure or glycaemic control but appears rather associated with an improved hypoxia, oxidative stress and/or AGE formation (Table 1). The rather heterogeneous list of potential contributors to diabetic nephropathy is tentatively integrated in a hypothetical scheme depicted in Figure 1. Clearly, the interrelationships among these various elements preclude the identification of a single culprit in the genesis of diabetic kidney lesions.

To assess the respective contribution of each of these mediators, low-molecular-weight compounds were designed to interfere with each target molecule. It is indeed important to acquire tools to evaluate and confirm our hypotheses. The safety and disposal of these various compounds need documentation in order to allow the transfer of experimental results to clinical practice.

Hypoxia correction

Diabetic glomerular damage decreases the number of peritubular capillaries and thus oxygen diffusion to tubulointerstitial cells, leading to tubular dysfunction and fibrosis [9]. Chronic hypoxia has indeed been documented in the diabetic kidney [3,10].

Defence against hypoxia hinges upon the hypoxia-inducible factor (HIF) [11]. Its activation induces a broad range of genes (e.g. erythropoietin, VEGF, HO-1, 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 SHR/NDmcr-cp rats [12]. Although hypertension and metabolic abnormalities remained unchanged [13], cobalt reduced proteinuria as well as histological kidney injury. Expressions of HIF-regulated genes, including erythropoietin, VEGF and HO-1, increased whereas the renal expressions of TGF-beta 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...
Table 1. Summary of animal experiments in a hypertensive, type 2 diabetic rat with nephropathy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Colonic restriction</th>
<th>ACE inhibitor</th>
<th>CCB</th>
<th>Diabetic control</th>
<th>Pioglitazone</th>
<th>Insulin</th>
<th>Cobalt</th>
<th>R147176</th>
</tr>
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<tr>
<td>Coloric restriction</td>
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<td>ARB, angiotensin receptor blocker</td>
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<td>β-blocker</td>
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<td>Pioglitazone</td>
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</table>

**Fig. 1.** Therapeutic targets for diabetic nephropathy, predicted from the study of experimental animals. The experimental studies of animals have incriminated newer culprits, including hypoxia, oxidative stress, advanced glycation end products (AGEs), PAI-1, etc. This figure is a modification of the original figure 5 in reference [3].

PHD where HIF binds, were therefore developed [14]. The resulting correction of chronic hypoxia might protect the diabetic kidney, independently of metabolic status and blood pressure.

The recent demonstration [15] that both erythropoietin and VEGF independently accelerate diabetic retinopathy warrants caution. PHD has three isoforms and, fortunately, the respective role of each PHD has been elucidated. PHD2 primarily regulates angiogenesis and erythropoiesis [16,17]. In contrast, the specific disruption of PHD1 induces hypoxia tolerance by reprogramming basal oxygen metabolism [18]. A specific PHD1 inhibitor may therefore be an interesting candidate for future therapy in diabetic nephropathy.

**Inhibition of advanced glycation**

As already mentioned, ARB is a potent inhibitor of advanced glycation end-products (AGEs) [19,20], both *in vitro* and *in vivo*, able to protect the kidney. 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 (AT1R) and thus virtually no anti-hypertensive effect [21].

The inhibition of AGE formation, the AT1R 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 AT1R binding. Despite a minimal effect on blood pressure, it provided significant renoprotection in SHR/NDmcrcp as well as in Zucker diabetic fatty rats [21]. 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 was protected by similar AGE and oxidative stress inhibitory compounds [22,23].
Plasminogen activator inhibitor (PAI)-1

The disruption of the plasminogen activator inhibitor-1 (PAI-1) gene protects mice against diabetic nephropathy [24]. Mice lacking the PAI-1 gene escape obesity and insulin resistance [25]. 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 (SBDD), to obtain molecules able to bind this site and thus inhibit PAI-1 activity. Two novel, orally active, small molecule substances, TM5001 and TM5007, were identified [26]. In vitro, they specifically inhibited PAI-1 activity and the formation of a PAI-1/TPA 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 [27]. 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.

Conclusion

The future prevention of diabetic nephropathy and of its dramatic consequences will undoubtedly rely on a multi-pronged approach. In addition to the current therapies insufficient to fully prevent renal complications, novel agents able to interfere with several newer culprits (e.g. those delineated in Figure 1) should provide additional, well-needed benefits. Only time will tell us if renewed approaches predicted from the study of experimental animals suffice in human.

Conflict of interest statement. None declared.

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