LEARNING THE ROPES:

Angiotensinogen suppression: a promising approach

LIWEI REN^{1,2} AND A.H. JAN DANSER¹

¹Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands ²Translational Medicine Collaborative Innovation Center, The Second Clinical Medical College (Shenzhen People's Hospital) of Jinan University, Shenzhen, China

DOI: 10.30824/2112-8

Hypertension remains the leading cause of death worldwide¹, despite the fact that more than 100 commercial drugs and drug combinations are available for its treatment. Blood pressure is often non-optimally controlled, either due to non-adherence or mechanisms that counteract the antihypertensive effect. For instance, in the case of angiotensin-converting enzyme inhibitors (ACEi) or angiotensin II type 1 (AT1) receptor blockers (ARBs), a renin rise will occur, due to interference with the negative feedback loop that normally allows angiotensin II to suppress renin. Here it is important to realize that, at 90% ACE inhibition, a 10-fold rise in renin is sufficient to restore the original angiotensin II levels. Such rises are easily achievable, and even renin rises of up to several 100-fold may occur. Hence, the idea has come up to combine multiple renin-angiotensin system (RAS) blockers at the same time to achieve better blockade. Unfortunately, this also resulted in more side effects. Another option is to target angiotensinogen, i.e., the ultimate source of all angiotensins. Normally, its levels are in the order of 1200 nmol/L, or almost 1 million times the level of angiotensin II. This allows angiotensin generation to rise in parallel with renin, even if renin increases 100-fold. Obviously, in the absence of sufficient angiotensinogen, renin will still rise substantially, but now it cannot generate angiotensins. Thus, this approach should prevent the normal counterbalancing consequences of the renin rise².

Circulating angiotensinogen is predominantly liver-derived, although several studies suggest that angiotensinogen is also generated at extrahepatic sites, like the kidney and adipose tissue. An elegant way of suppressing angiotensinogen is to make use of antisense oligonucleotides (ASO)³ or small interfering RNA (siRNA)⁴, particularly when conjugated to the

hepatocyte-targeting ligand N-acetylgalactosamine (GalNAc). This ligand recognizes the asialoglycoprotein receptor, which is primarily expressed on hepatocytes. Upon binding it will be rapidly endocytosed, and once intracellular both ASO and siRNA ultimately result in the destruction of angiotensinogen mRNA, thereby preventing de novo synthesis of angiotensinogen. Due to their liver-specificity, low doses of GalNAc-conjugated siRNA or ASO are sufficient to suppress angiotensinogen⁵. It is worth noting that several liver-targeted siRNA drugs (e.g., patisiran, for hereditary transthyretin amyloidosis, and inclisiran, for lipid lowering) already exist. Remarkably, their half-life is exceptionally long, allowing a dosing regimen of at most 2-3 injection per year⁶.

Angiotensinogen siRNA and ASO are now being evaluated in phase 1/phase 2 clinical trials. To obtain a better understanding of their mechanism of action, we have recently investigated the effects of angiotensinogen siRNA in spontaneously hypertensive rats (SHR) and in rats with chronic kidney disease (CKD) following 5/6th nephrectomy^{4,7}. In both models, siRNA lowered circulating angiotensinogen by >95%. As expected, in SHR renin rose considerably after this treatment. This allowed circulating angiotensin II levels to remain intact, while the renal levels of angiotensin II dropped by >50%. The latter indicates that it is particularly tissue angiotensin production that is sensitive to the siRNA approach. Remarkably, adding an ARB on top of siRNA in SHR led to an even further rise in renin, and now angiotensinogen became depleted, so that angiotensin II virtually disappeared, both in blood and kidney. The blood pressure-lowering effect of siRNA in SHR was comparable to that of an ARB or ACEi (Figure 1A), while in combination with an ARB blood pressure dropped

synergistically, reflecting the virtual annihilation of angiotensin II. Under these conditions kidney function (glomerular filtration rate) remained intact, while cardiac hypertrophy decreased in parallel with blood pressure.

The 5/6th nephrectomy CKD model is characterized by low renin levels, and the blood pressure-lowering effects of siRNA in this situation was therefore modest. It could not be enhanced by concomitant treatment with an ARB. No rises in renin occurred. Nevertheless, siRNA improved proteinuria and reduced glomerulosclerosis, and the reduction in renal angiotensin II content after siRNA turned out to be an independent determinant of this renoprotective effect (Figure 1B). Like in SHR, siRNA in the CKD rat model improved cardiac hypertrophy, while it did not affect glomerular filtration rate. Importantly, in both models, renal angiotensinogen virtually disappeared after liver-specific angiotensinogen siRNA treatment. This demonstrates that renal angiotensinogen, despite the occurrence of angiotensinogen mRNA in the kidney (which was unaffected by siRNA), is of hepatic origin after all.

These data indicate that angiotensinogen siRNA lowers blood pressure to the same degree as classical RAS blockers, yet at a much lower dosing frequency. Given its stable and sustained efficacy, lasting weeks, RNA interference may offer a unique approach to improving therapy adherence and treat hypertension. In addition, angiotensinogen siRNA exerts renoprotection in a blood pressure-independent manner, by specifically reducing renal angiotensin II levels, based on the fact that renal angiotensin generation relies on hepatic angiotensinogen. This may involve megalin-mediated reabsorption of filtered angiotensinogen in the proximal tubule⁸.

Although the long-lasting effects of the siRNA approach might be considered as an advantage, it also poses a threat, for instance in women becoming pregnant during treatment, or in cases of emergency, when severe hypotension occurs, and the RAS is needed. Here novel tools ('REVERSIR') are now being developed, capable of acutely reversing the effect of siRNA⁹. This concerns short, synthetic, high-affinity oligonucleotides complementary to the siRNA guide strand, that can be targeted to the liver making use of the same GalNac approach. Finally, given the liver accumulation of the siRNA, one might speculate about liver toxicity, or inflammatory and immunological side effects. Yet, no such observations were made for liver-targeted proprotein convertase subtilisin/kexin type 9 siRNA (inclisiran) over a 6-month

period¹⁰. Since this siRNA relies on the same GalNac principle, at present the safety profile of such drugs seems excellent.

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SHR

CKD

Figure 1. Panel A, effect of angiotensinogen siRNA, valsartan, captopril or their combination on mean arterial blood pressure (MAP) and circulating angiotensin I and II levels in spontaneously hypertensive rats (SHR). Panel B, effect of angiotensinogen siRNA, losartan, captopril or their combination on circulating angiotensin I and II, renal angiotensin II and proteinuria in rats with chronic kidney disease (CKD). Data are from references 4 and 7. * $P < 0.05$, ** $P < 0.01$, and **** $P < 0.0001$ vs. vehicle.

Liwei Ren - l.ren@erasmusmc.nl

Jan Danser - a.danser@erasmusmc.nl