Renal Angiotensinogen Is Predominantly Liver Derived in Nonhuman Primates

Masayoshi Kukida, Lei Cai, Dien Ye, Hisashi Sawada, Yuriko Katsumata, Michael K. Franklin, Peter I. Hecker, Kenneth S. Campbell, A.H. Jan Danser, Adam E. Mullick, Alan Daugherty, Ryan E. Temel, Hong S. Lu

AGT (angiotensinogen) is the unique substrate of the renin-angiotensin system. While many cells synthesize AGT, studies in mice have demonstrated that plasma AGT is predominantly liver derived. Indeed, either pharmacological inhibition or genetic deficiency of hepatocyte-derived AGT reduces blood pressure and atherosclerosis in mice. Locally synthesized AGT in the kidney may contribute to renal Ang II (angiotensin II) generation, possibly in a blood pressure–independent manner, although the literature is inconsistent. Based on this concept, AGT measurements in urine or renal biopsies are used often as an independent marker of the renal renin-angiotensin system activation in humans. However, it remains unclear whether renal Ang II generation in humans depends on kidney-derived AGT.

AGT is cleaved by renin into 2 products: Ang I (angiotensin I), which consists of 10 amino acids, and des(Ang I)AGT, which has 443 amino acids in mice and 442 amino acids in humans and nonhuman primates (NHPs). Although sequences of AGT vary substantially between mouse and human, this protein is highly conserved in humans and NHPs. Plasma total AGT concentrations were 3 to 4 μg/mL in mice, 15 to 41 μg/mL in humans, and 11 to 20 μg/mL in cynomolgus monkeys. Plasma AGT was predominantly present as des(Ang I)AGT in mice (≈92%) in contrast to both humans and cynomolgus monkeys (<40% of des(Ang I)AGT). Despite differences in plasma AGT, the distribution of AGT protein accumulation within the kidney was comparable among the three species. AGT protein accumulation was most abundant in the renal proximal convoluted tubules (S1 and S2 segments), modest in the proximal straight tubules (S3 segment), and not detected in glomeruli and other tubules of the kidneys (Figure [A]).

Given the similarity between humans and cynomolgus monkeys, findings in the latter likely have greater translational significance, compared with rodent models, in defining the origin of kidney AGT in humans. To determine whether liver-derived AGT contributes to AGT protein accumulated in the kidney of NHPs, female cynomolgus monkeys (3–4 years of age) were injected subcutaneously with either saline or antisense oligonucleotides (ASOs) targeting liver-derived human AGT (Ionis; GalNAc AGT ASO: conjugated with N-acetyl galactosamine; 2.5 or 10 mg/kg). This human GalNAc AGT ASO has an identical sequence match to AGT mRNA in cynomolgus monkeys. Saline or ASO was injected on days 1 and 4 and then once weekly for a subsequent 4 weeks. Neither dose of ASO affected body weight or liver and kidney functions.

Both doses of GalNAc AGT ASO reduced plasma AGT concentrations within 1 week by up to 80% (Figure [B]). Liver had ≈160-fold more AGT mRNA abundance than kidney and visceral adipose tissue (Figure [C]). Both doses of GalNAc AGT ASO profoundly reduced hepatic mRNA abundance of AGT (Figure [C]). The low dose (2.5 mg/kg) of GalNAc AGT ASO did not affect renal AGT mRNA abundance, whereas the high dose (10 mg/kg) reduced renal AGT mRNA abundance (Figure [D]). Of note, both doses of GalNAc AGT ASO produced equivalent reductions in both plasma (Figure [B]) and liver AGT (Figure [C]). This illustrates that ASO can be detected...
Figure. Contributions of liver-derived AGT (angiotensinogen) on renal renin-angiotensin system in cynomolgus monkeys.

A, Immunostaining of AGT in kidney sections from mice, humans, and cynomolgus monkeys using a rabbit anti-mouse AGT antibody for mice (IBL America 28101; 0.3 μg/mL) and a mouse anti-human AGT antibody for both humans and monkeys (IBL America 10417; 1 μg/mL). B–F, Female cynomolgus monkeys were injected subcutaneously with either saline (indicated as antisense oligonucleotide [ASO] 0) or GalNAc AGT ASO (2.5 or 10 mg/kg; indicated as ASO 2.5 and ASO 10, respectively) for 5 wk (n=4/group). B, Plasma total AGT concentrations were determined by an ELISA kit (IBL America 27412). Data are represented as mean±SEM. Piecewise linear mixed model with a split point at week 1 was used to compare plasma AGT concentration changes over time among the three groups. (Continued)
in kidney and exhibits some activity at sufficiently high doses such as 10 mg/kg in cynomolgus monkeys. Irrespective of renal AGT mRNA, both doses of GalNAc AGT ASO diminished renal AGT protein accumulation to a similar extent (Figure [D]). Plasma renin activity was not altered by either dose of GalNAc AGT ASO (Figure [E]), consistent with the recent data evaluating IONIS-AGT-LRX in hypertensive patients. These data imply that renin upregulation must have matched AGT downregulation to keep angiotensin generation in the normal range. As shown by immunostaining, diminished AGT protein accumulation following dosing with GalNAc AGT ASO was noted in the S1 and S2 segments of renal proximal tubules (Figure [F]). These findings support the notion that liver supplies the bulk of AGT protein to the kidney in NHPs, independent of the presence of renal AGT mRNA. The Figure (F) also shows the distribution of other renin-angiotensin system components. Renin was observed predominantly in juxtaglomerular cells, and ACE (angiotensin-converting enzyme) and ACE2 were present in all 3 segments of the proximal tubules, being most abundant in the S3 portion. GalNAc AGT ASO did not change the renal distribution of these enzymes.

In conclusion, the liver is the major source of AGT in kidneys of cynomolgus monkeys. Hepatic AGT accumulation in the S1 and S2 segments of the proximal tubules coincides with the observation in humans that tubular reabsorption via megalin—an endocytic receptor on the proximal tubules—is the main determinant of urinary AGT. Taken together, these data are consistent with renal AGT originating predominantly in the liver. This implies that renal Ang II production in NHPs and humans relies on hepatic AGT and that the concept that AGT in urine or renal biopsies reflects an independent renal renin-angiotensin system needs to be reconsidered.

**REFERENCES**


**ARTICLE INFORMATION**

**Affiliations**

Saha Cardiovascular Research Center (MK, LC, D.Y., H.S., MKF, PIH, AD, RET, HSL), Saha Aortic Center (H.S., AD, HSL), Department of Physiology (H.S., KSC, AD, RET, HSL), Department of Biostatistics (YK), and Sanders-Brown Center on Aging (YK), University of Kentucky, Lexington, Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands (D.Y., AJHJD), Ionis Pharmaceuticals, Carlsbad, CA (AEM).

**Acknowledgments**

We thank the CCTS Biospecimens Core (supported by UL1TR001998) at the University of Kentucky for providing human samples.

**Sources of Funding**

This project was supported by the National Institutes of Health grants R01HL139748 and R01HL111932.

**Disclosures**

A.E. Mullick is an employee of Ionis Pharmaceuticals, Inc. The other authors report no conflicts.