OF DIABETIC MICE AND ACE2: A NEW BIOMARKER OF RENAL DISEASE?

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Diabetic nephropathy is a leading cause of progressive renal failure and mortality in an increasing number of patients, as well as a key risk factor in cardiovascular disease, and constitutes an accelerating burden on the healthcare system. Blockade of the renin-angiotensin systems (RAS) continues to be an effective therapeutic strategy to attenuate diabetic renal injury and the decline in renal function. The current RAS therapies target either the angiotensin-converting enzyme (ACE) to block the conversion of ANG I to ANG II, or the AT1 subtype receptor antagonists (ARB) to prevent ANG II-AT1 receptor (AT1R) signaling. Both of these approaches impact other intrinsic pathways of the RAS, including the activation of the ACE2-ANG-(1–7)-AT7/Mas receptor (AT7R) axis that antagonizes the actions of ANG II (3). The carboxypeptidase ACE2 is now recognized as a key component of this system that efficiently converts ANG II to ANG-(1–7) in a single hydrolytic step and may alter the functional output of the RAS (3). Indeed, knockdown of ACE2 or administration of an ACE2 inhibitor exacerbated the degree of renal injury in several diabetic models and reduced the therapeutic benefit of ACE inhibition that likely reflects increased ANG II and lower levels of ANG-(1–7) within the kidney (10, 12). The current study by Wysocki et al. (13) in the American Journal of Physiology-Renal Physiology established the enhanced excretion of ACE2 in both type 1 (streptozotocin-induced) and type 2 (db/db strain) diabetic models and reveals the potential role of the enzyme as a urinary biomarker of diabetic injury and/or activation of the intrarenal RAS. The authors report that the proximal tubules of the kidney are the primary source for increased excretion of ACE2 and that the urinary enzyme comprises both 110- and 75-kDa molecular forms. Although circulating ACE2 is normally low or nondetectable, serum levels are increased in both experimental and human diabetes (11–14). The present study clearly demonstrates that infusion of a recombinant soluble form of ACE2 does not influence urinary levels of the enzyme, suggesting the distinct compartmentalization of circulating and renal/urinary ACE2.

Importantly, the present results add to the emerging view that renal pathologies may not be due to a deficit in ACE2 per se but that increased expression of the enzyme may reflect a compensatory tubular response to alter the ANG II/ANG-(1–7) balance within the kidney. Exacerbation of renal injury by ACE2 blockade in diabetic or hypertensive conditions may be particularly evident as the tubular epithelium attempts to buffer the ANG II-AT1R axis and the stimulation of fibrotic and inflammatory pathways. The study also suggests that the increase in urinary ACE2 reflects the enhanced release or shedding of the peptidase into the tubular fluid and its potential use as a biomarker of diabetic injury. However, it is not known whether the shedding of ACE2 from the apical face of the proximal tubule is ultimately beneficial or deleterious to the kidney. This report and others find that the soluble form of ACE2 (110–120-kDa species) is catalytically active and hydrolyzes ANG II to ANG-(1–7) similar to the membrane-bound form (4, 9). Given the high catalytic efficiency of ACE2...
for ANG II, we presume the enzyme is localized on the apical membrane in close proximity to the AT1R to facilitate the metabolism of ANG II and terminate a sustained activation of the receptor (Fig. 1). Thus ACE2 shedding may impair the ability of the peptide to effectively metabolize ANG II at the ectocellular side of the apical surface. Moreover, the soluble form of the peptide may be more sensitive to degradation and inactivation in comparison to the membrane-anchored enzyme. Indeed, the authors find a single protein band for ACE2 in the renal tissue but two forms of 110 and 75 kDa in the urine (13). Unfortunately, the current ACE2 knockout models or treatment with the nonpeptide ACE2 inhibitor MLN4760 will not distinguish between the membrane-bound and soluble forms of the enzyme to peptide metabolism.

An interesting aspect of the current study is the lack of an effect by the ACE inhibitor or AT1R antagonist to influence the urinary excretion or tissue levels of ACE2 in the db/db mice despite the reduction in albuminuria. One caveat with these experiments is the relatively short duration of treatment with RAS inhibitors and that long-term inhibition may be required to influence ACE2 expression and/or release. The authors did not evaluate whether markers of tubular injury such as kidney injury marker 1 (KIM-1) or N-acetylglucosidase (NAG) excretion were influenced by RAS blockade. Future studies should assess the extent that ACE2 excretion correlates with markers of tubular damage prior to and following blockade of the RAS.

In this aspect, Elased and colleagues (4) also report increased urinary excretion of ACE2 in type 2 diabetic db/db mice, and that the insulin-sensitizing agent rosiglitazone reduced urinary excretion of ACE2 in type 2 diabetic db/db mice, and that the insulin-sensitizing agent rosiglitazone reduced urinary but not tissue levels of the enzyme. A reduction in the shedding of ACE2 and maintenance of the tubular levels of the peptidase may constitute an additional benefit to the overall effects of this therapeutic agent. The Elased study localized ACE2 and the putative ACE2 secretase disintegrin and metalloproteinase 17 (ADAM17) to the apical aspect of the proximal tubules; the enhanced expression of ADAM17 in db/db mice was also attenuated with rosiglitazone (4). Lambert et al. (7) originally reported that ADAMs contribute to the release of ACE2 from MDCK cells. A subsequent study by McCray and colleagues (6) found that ADAM17 mediated ACE2 shedding from pulmonary epithelial cells, as well as identified a specific sequence in the juxtamembrane region of ACE2 recognized by the secretase.

The mechanism that contributes to the shedding of ACE2 in the diabetic kidney remains unresolved at this time; however, the potential role of ADAM17 to facilitate release of ACE2 may well contribute to an array of mechanisms that underlie tubular injury. As shown in Fig. 1, the activation of ADAMs on the apical surface stimulates the release of tethered ligands including TNFα, EGF, and TGFβ that bind and activate their respective receptors (5). Provided that ADAMs indeed influence ACE2 release from the tubular membrane, local concentrations of ANG II may increase, leading to an enhanced stimulation of AT1R-dependent events. Moreover, EGFR transactivation is a canonical signaling event downstream from the ANG II-AT1R pathway within the kidney and other tissues (8). In turn, the enhanced shedding of ACE2 may also reduce expression of ANG-(1–7), a peptide that antagonizes the ANG II-AT1R axis by the formation of nitric oxide and activation of cellular phosphatases (Fig. 1). Benter and colleagues find that ANG-(1–7) attenuated EGFR activation via the MAP kinase pathway in response to ANG II, as well as reduced the degree of renal injury in the diabetic spontaneously hypertensive rat (1, 2). Thus the increased expression of ADAMs may portend for the enhanced shedding of ACE2, activation of the ANG II-AT1R pathway, and the release of various cytokines and growth factors within the diabetic kidney. The assessment of ACE2 expression or activity in the urine as proposed in the current study may well serve as a biomarker of early diabetic events that can lead to tubular injury and reduced renal function. As to whether the shedding of ACE2 in the tubular epithelium compromises the best laid plans of the kidney to attenuate the development and progression of diabetic injury, this issue awaits another chapter in the continuing story of the RAS and renal disease.

REFERENCES


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DISCLOSURES

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AUTHOR CONTRIBUTIONS

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