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Reduced plasma ACE2 activity in dialysis patients: another piece in the conundrum of factors involved in hypertension and cardiovascular morbidity?

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The renin–angiotensin system (RAS) is a complex regulatory network consisting of enzymes and effector peptides that facilitate the maintenance of homeostasis of several physiological processes [1, 2]. Upregulation of the RAS system, however, under chronic conditions and mainly through the interactions of Ang II with the AT1 receptor, can lead to hypertension and is involved in the progression of diabetic and nondiabetic chronic kidney disease (CKD) [2, 3]. Clinical interventions targeting the RAS and its pathogenic actions have been centered on the use of RAS blockers [3]. More recently, it has been proposed that the RAS system could be therapeutically targeted by increasing Ang II degradation and Ang (1–7) formation via ACE2 enzyme activation [2, 4–7]. ACE2, a monocarboxypeptidase discovered in 2000, metabolizes Ang II by removing a single amino acid, phenylalanine, from the C-terminus of this peptide [8], which results in the formation of Ang-(1–7) [8, 9]. Angiotensin-(1–7) has anti-inflammatory and antiproliferative actions that tend to counteract the proinflammatory and pro-proliferative effects of Ang II.

ACE2 is a type-1 integral membrane glycoprotein [10] that is expressed largely in the kidney, and the intestine, but is also present in the heart, lungs and brain and is more ubiquitous than initially thought [2]. In its full-length form, ACE2 consists of three structural entities: the cytosolic, transmembrane and extracellular domains and has a molecular weight of 120–130 kD [11–15]. The extracellular domain of ACE2, which confers its enzymatic activity, contains a single catalytic metallopeptidase unit that shares 42% sequence identity and 61% sequence similarity with the catalytic domain of ACE [8]. Notably, pharmacologic ACE inhibitors used in clinical practice do not inhibit ACE2 [8].

In this issue of Nephrology Dialysis Transplantation, Roberts *et al.* [16] showed that among patients with CKD plasma ACE2 activity is lower in those undergoing hemodialysis for end stage renal disease (ESRD) when compared with predialysis patients with CKD or renal transplant patients. When compared with historic samples from healthy subjects, however, all CKD groups examined, i.e. predialysis, transplant patients and even subjects on dialysis, seemed to have increased levels of plasma ACE2 activity. Statistical comparisons with healthy controls, however, could not be done because samples from healthy individuals were not assayed concurrently with those in the present study. What the study shows, in our opinion, is that while plasma ACE2 activity tends to increase in CKD patients, perhaps as a compensatory mechanism to attenuate Ang II overactivity, at the time that ESRD is reached and dialysis initiated a relative deficiency in plasma ACE2 activity ensues.

What is then the significance of reduced plasma ACE2 activity in dialysis patients? As ACE2 is mainly involved with the degradation of Ang II, one could readily speculate that the levels of this peptide could be augmented thereby predisposing to hypertension and other cardiovascular morbidity. As the levels of Ang II or Ang (1–7) were not measured in this study, one can only consider this as a predictable consequence of ACE2 deficiency that, however, still needs to be demonstrated. One also wonders about the significance of plasma ACE2 activity since ACE2 is mainly a tissue enzyme and its levels in the circulation, unlike the levels of ACE, are relatively low. Initial attempts to measure ACE2 directly in plasma from healthy individuals were unsuccessful [17, 18]. Moreover, circulating ACE2 enzymatic activity has also been shown to be low or even undetectable in animals under physiological conditions [19–22]. Interestingly, in pathological states in humans, such as ischemic heart disease [23], heart failure [24] and diabetes accompanied by vascular complications [25] as well as in rodent models of diabetes [19, 26] circulating ACE2 activity is augmented. Other studies in patients with connective tissue diseases, by contrast, have reported antibodies against plasma ACE2 that reduce enzymatic activity [27].

The intriguing observation that human plasma itself may inhibit ACE2 enzymatic activity was made based on incubation of purified recombinant ACE2 with human plasma [18]. These findings suggested the presence of a small molecular endogenous inhibitor of ACE2 in human plasma. Consistent with this, Roberts *et al.* now show that in a small subset of patients from each CKD subgroup, ACE2 activity in unprocessed plasma was markedly lower than in plasma samples that had undergone the extraction process to remove the endogenous inhibitor of ACE2 [16].

One wonders whether the process of hemodialysis itself could alter the levels of the ACE2 inhibitor in plasma owing to its small molecular size. Studies before and after the dialysis procedure could be informative in this regard. In the study by Roberts *et al.* [16], samples from patients on hemodialysis were collected prior to commencing dialysis, and on the middle dialysis day of the week. Removal of the inhibitor during the dialysis procedure, however, could only increase plasma ACE2 activity which is the opposite of what was observed in dialysis patients. The results of this study can therefore be interpreted to signify that the extraction of the endogenous inhibitor by dialysis is not a cause of reduced plasma ACE2 activity. A possibility that the small-molecular inhibitor of ACE2 might form a complex with ACE2 protein thereby evading extraction during dialysis needs to be considered.

If the plasma ACE2 inhibitor indeed does not play a role, the question that remains is what causes the observed reduction in plasma ACE2 activity in patients with ESRD undergoing dialysis. The source of circulating ACE2 in healthy individuals and CKD patients is not clear but release from the kidneys is a possibility. The levels of ACE2 activity have been found to be 10- to 30-fold higher in mouse kidney cortex than in the heart [22] and urinary ACE2 activity is about 10-fold higher in urine than in plasma [28]. The mechanism of how ACE2 reaches the circulation and is then released into plasma is not well understood and requires further examination. It has been proposed that soluble ACE2, which lacks its cytosolic and transmembrane domains, arises from proteolytic 'shedding' of the membrane-bound enzyme [15]. It is, nevertheless, conceivable that the full-length, membrane-bound ACE2 can also be released into plasma, especially in disease states associated with tissue damage, such as myocardial infarction.

In cell culture experiments, shedding of soluble ACE2 is stimulated by a disintegrin and metalloproteinase, ADAM17, [15] and inhibited upon interaction of ACE2 with calmodulin [29]. Unlike ACE, which is an endothelial enzyme, ACE2 is normally not expressed or only minimally expressed in the endothelial layer [30]. It is possible, however, that under pathologic conditions, there may be an aberrant neoexpression of ACE2 in endothelial cells [31]. This abnormally expressed endothelial protein could be shed into the circulation and could be the source of increased plasma ACE2 activity in certain conditions such as myocardial infarction, kidney disease or diabetes. There have been no studies, to our knowledge, that examined plasma ACE2 and the possible pathways such as ADAM17 or calmodulin that might be involved in modulating the release of this enzyme into the circulation.

As the *ACE2* gene is located on chromosome X, possible differences in plasma ACE2 activity between the sexes were examined by Roberts *et al.* [16] in a multivariate analysis for males and females separately. The predictors of plasma ACE2 activity in patients undergoing dialysis appeared to be different in both sexes. While in males plasma ACE2 activity was strongly associated with BNP, which is increased in left ventricular hypertrophy and systolic dysfunction, female patients undergoing hemodialysis showed significant associations with diabetes and postdialysis systolic blood pressure. Whether these associations have pathogenic or therapeutic implications awaits further examination. Moreover, the authors found that female hemodialysis patients compared with male counterparts had lower plasma ACE2 activity. Analogous trend of lower plasma ACE2 activity in the female group was observed for kidney transplant patients, which is similar to the recent findings of Soler *et al.* [23] who found plasma ACE2 activity significantly lower in female transplant patients when compared with males.

In summary, the article by Roberts *et al.* suggests that, in patients with CKD, plasma ACE2 activity is increased whereas in patients with ESRD undergoing dialysis, by contrast, plasma ACE2 activity is reduced when compared with predialysis CKD patients. Whether ACE2 in plasma is indeed altered in CKD patients needs to be confirmed in further studies that include contemporary measurements from healthy control subjects. Moreover, a longitudinal follow-up of a CKD cohort would be ideal to monitor ACE2 activity as renal function declines over time. Several other questions remain unanswered, such as the mechanism driving ACE2 into circulation in disease states. Regardless of the mechanism, the reduction in plasma ACE2 activity reported in ESRD patients treated by dialysis, particularly in female subjects, could limit Ang II degradation leading to increased levels of this peptide which could contribute to the high prevalence of hypertension [32] and cardiovascular morbidity [33, 34] that afflicts the dialysis population.

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CONFLICT OF INTEREST STATEMENT

The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Health. None declared.

(See related article by Roberts *et al.* Angiotensin-converting enzyme 2 activity in patients with chronic kidney disease. *Nephrol Dial Transplant* 2013; 28: 2287–2294.)

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