IN FOCUS

The importance of uromodulin as regulator of salt reabsorption along the thick ascending limb

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Uromodulin (UMOD), also named Tamm Horsfall protein, is the most abundant protein secreted in the urine under normal conditions. It was purified the first time in 1950 and since then considerable efforts have highlighted its importance in human pathophysiology. However, its precise biological functions still remain elusive. The clinical interest in UMOD derives from the evidence that UMOD genetic mutations result in tubulointerstitial nephropathies currently known as UMOD-associated kidney disease (UAKD), rare genetic disorders characterized by hyperuricaemia, gout and a progressive decline of renal function [1]. In addition, UMOD has been proposed to modulate water and electrolyte homeostasis by acting on the main transporters expressed along the thick ascending limb (TAL) and the early distal convoluted tubule. The precursor undergoes extensive post-translational modifications through the endoplasmic reticulum (ER) and the Golgi apparatus, and ultimately it is targeted to the apical membrane [2]. From the luminal site, it is cleaved by an unknown protease and then released into the tubular fluid.

The exact mechanism linking UMOD mutations to renal concentrating defect has not been fully characterized. It has been suggested that the filamentous gel-like structure of the extracellular domain may serve as a barrier to water permeability [3]. In vitro and in vivo studies have recently demonstrated that UMOD modulates the function of the Na-K-2Cl (NKCC2) co-transporter [4] and the renal outer medullary potassium (ROMK) channel [5] (Figure 1). UMOD-deficient mice showed normal electrolyte balance at basal and urine concentrating defect after water deprivation [6]. Impaired urine concentration was coupled with a compensatory up-regulation of distal Na⁺ transporters, including the Na-Cl co-transporter (NCC), suggesting indirectly an impaired function of the TAL. Immunostaining analysis revealed the absence of any difference in NKCC2 protein abundance on the apical membrane between knockout (KO) and wild-type (WT) mice, but an increased sub-apical immunoreactivity, with overall increased NKCC2 protein abundance compared with WT [4]. It is possible that an impaired protein degradation, in the absence of UMOD, resulted in NKCC2 accumulation. However, the same study demonstrated that phospho-NKCC2 levels, a marker of NKCC2 activity, were lower in KO than WT mice, and intraperitoneal injection of frusemide resulted in attenuated natriuretic and cloruretic responses, further supporting the hypothesis of reduced NKCC2 activity in the absence of UMOD. Consistent with these findings, in vitro NKCC2 phosphorylation was enhanced in the presence of UMOD, indicating that also in cultured cells UMOD promoted NKCC2 activity. This hypothesis has been further corroborated by recent observations linking salt-sensitive hypertension with a genetic UMOD variant leading to an increased UMOD synthesis and secretion in humans [7]. Transgenic mice expressing this genetic variant resembled human features, showing salt-sensitive hypertension. This finding correlated with the up-regulation of NKCC2, with increased protein phosphorylation via the STE20/SPS1-related proline/alanine-rich kinase (SPAK) and the down-regulation of the negative regulator kidney-specific KS-SPAK. Besides NKCC2, UMOD has been shown to modulate also the activity of ROMK. Renigunta et al. [5] have shown that UMOD co-localized with ROMK in a protein lysate from mice, while in oocytes, co-expression of UMOD and ROMK resulted in increased current amplitude, associated with an increased surface ROMK abundance.

Patients suffering from UAKD commonly do manifest a defect in urine concentrating ability even before the decline of the GFR [8]. The mechanism by which UMOD mutations lead to urine concentrating defect in humans remains to be better elucidated.

In this issue of NDT, Labriola et al. [9] show original data exploring the tubular function of a patient suffering UAKD during the early phase of the disease.

The authors compared the response to frusemide, water restriction and dDAVP tests in a 32-year-old woman holding a pathogenetic mutation of UMOD with her non-affected sister. Following frusemide administration, the patient showed a more significant reduction in the body weight and blood pressure, larger urine output and fractional Na⁺ excretion (FE %) than the control. This exaggerated response to frusemide looks surprising. Patients with a known dysfunction of the TAL, such as Bartter syndrome patients, show a variable response to frusemide infusion, depending on genetic mutation. Those
carrying mutations in SLC12A1, encoding the Na-K-2Cl co-transporter NKCC2, show no response to frusemide, while patients with KCNJ1 and CLCNKB mutations have a normal response [10], presumably because of a less severe defect in salt absorption. UMOD KO mice showed an attenuated response to frusemide administration, suggesting a defect in NaCl absorption along the TAL. The paradoxical response of the UAKD patient to frusemide suggests certainly the presence of a residual function of the TAL. The authors interpreted their findings as the result of a hyper-activation of residual NKCC2, which in the early phase of the disease may be sufficient to ensure normal sodium and water balances at basal. The use of frusemide resulted in the disruption of this equilibrium, with an exaggerated response, for a presumable lack of adequate distal compensation acutely. This intriguing interpretation requires further studies to be confirmed.

The most important limitation of the study, as the authors admitted, is the low number of patients. The test has been conducted only in one patient, so firm conclusions may be made, as an underlying inter-individual variability may account for the difference in the magnitude of the response. In addition, the opposite effect of frusemide in the patient compared with UMOD KO mice may depend on the impact of the genetic mutation on protein function. The majority of mutations leading to human UAKD have been shown to affect protein folding with ER retention [11]. Schaeffer et al. [12] have shown that part of mutant UMOD escapes ER retention, reaches plasma membrane and is secreted in the urine, while in UMOD KO mice the protein is completely absent. The patient’s mutation may potentially activate NKCC2, as a result of a gain of function mutation, thus explaining the exaggerated response to frusemide. In fact, the reduced NKCC2 protein abundance in the renal biopsy of UAKD patients has been shown only in the late stage of the disease and may reflect general tissue derangement occurring during the late stage of kidney disease.

The authors explored further the function of the TAL by performing the water restriction followed by the desmopressin (dDAVP) test. The patient showed a preserved response to water deprivation. Interestingly, dDAVP administration failed to induce a significant elevation of urine and plasma osmolality in the patient. The mechanism underlying this defect has not been further investigated.

Urine concentration is the result of a combined function of the collecting duct (CD) and the TAL, in the presence of vasopressin (AVP) [13]. Endogenous or exogenous AVP enhances water absorption along the CD from the tubular fluid through an increased water channel Aquaporin 2 (AQP2) protein abundance on the apical membrane. The TAL contributes to the urinary concentrating process by generating a medullary osmotic gradient, through the active salt reabsorption.

The function of the CD as well as AVP plasma levels were intact in an animal model resembling UAKD; conversely, histological lesions originating from the TAL could predict a dysfunction of this segment leading to the loss of medullar hypertonicity [14]. The authors postulated that the blunted response to dDAVP could result from (i) a failure to generate the interstitial osmotic gradient, because of the reduction of NaCl absorption and the reduced water impermeability of the TAL and (ii) the lack of dDAVP-dependent NKCC2 stimulation, as observed in UMOD KO mice [4]. Further studies are needed to better explore the mechanism underlying this defect in humans.

In this paper, the patient had undergone dDAVP administration after 6 h of water restriction. In this condition, endogenous AVP is stimulated. To better evaluate the maximal response to dDAVP, it would be more appropriate to administer the drug after a water load, to suppress endogenous AVP. Then, measuring urine AQP2 and NKCC2 excretion may help to confirm that the function of the CD is intact also in humans and that the NKCC2 response is blunted.
In conclusion, the results of this interesting study give more insight into the mechanism underlying renal damage in UAKD, suggesting a mild dysfunction of the TAL, compensated in basal conditions, and unmasked after frusemide infusion during the early stage of the disease. Later, the reduction of the renal function is coupled with a clear decline of NKCC2 staining in renal biopsy, and a more diffuse damage of renal parenchyma, predicting the evolution to the end stage renal disease.

CONFLICT OF INTEREST STATEMENT

None declared.


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Evidence-based choice of dialysis technique in diabetics with end-stage kidney disease: half a loaf is better than no bread

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MANY STUDIES, MANY PATIENTS, AND SCARCE EVIDENCE

In this issue of the journal, Couchoud and members [1] of the ERBP Diabetes Guideline Development Group have performed a very comprehensive review on research papers comparing haemodialysis (HD) and peritoneal dialysis (PD) in patients with diabetes. The aim of the paper was to review available evidence to develop specific guidelines for patients with diabetes and end-stage kidney disease (ESKD): this is an important document for the nephrology community because it addresses the issue of the best dialysis therapy for patients with the most frequent cause of renal failure worldwide. A very laconic way to summarize the paper would be to say that no