WATER AND SALT

SO030 A MOUSE MODEL OF SALT WASTING, HYPERCALCIURIA AND KIDNEY STONES

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Introduction and Aims: Calcium nephrolithiasis is a complex disease with multiple pathogenetic mechanisms. The role of salt wasting and the subsequent volume depletion in the pathogenesis of hypercalciuria and kidney stone formation remains speculative. The only known monogenic disorder associated with salt wasting/volume depletion, hypercalciuria and nephrolithiasis/ nephrolithoclastis is Bartter Syndrome. We have developed a model of distal tubule salt wasting, which is caused by the simultaneous deletion of the CT/HCO3-exchanger pendrin and the Na-C1 co-transporter NCC (PNAS 2012). These animals become severely volume depleted and have nephrogenic DI (PNAS 2012). We hypothesized that salt wasting followed by volume depletion, irrespective of the etiology or the nephron segment(s) involved, activates a cascade of events that lead to increased generation of the arachidonic acid metabolites Prostaglandin E2 (PGE2) and 20-hydroxyeicosatetraenoic acid (20-HETE) that impair salt and calcium reabsorption in the thick ascending limb and the proximal tubule, and block the action of AVP and aldosterone on water and salt reabsorption in the collecting duct, leading to the worsening of volume depletion and calcium wasting. We hypothesize that this self-propagating disadvantageous cycle can lead to super-saturation of calcium crystals in the urine and result in nephrolithiasis and/or nephrolithoclastis.

Methods: DNA microarray, northern hybridization, western blotting, immunofluorescence labeling, IHC and E staining, Von Kossa stain, functional studies and appropriate treatment with various chemicals were performed on kidneys of wt and double pendrin/NCC KO mice.

Results: DNA microarray demonstrated the activation of arachidonic acid metabolites PGE2 and 20-HETE-generating cytochrome p450 isoforms (Cyp4a12a and 12b) in kidneys of dKO mice. The 24 hr urine collection indicated significant increases in PGE2 and 20-HETE excretion in dKO mice (p<0.01 vs WT or single KO mice). In addition to profound salt wasting, the 24 hr urine analysis showed a 3-fold increase in calcium excretion in dKO mice. The histological analysis of kidneys demonstrated multiple calcium stones in the medullary collecting ducts in dKO mice but not in pendrin or NCC KO mice. The stones were comprised of calcium based on strong staining with Von Kossa stain. Phosphate excretion increased by 2 folds in dKO mice and correlated with a significant reduction in the expression of NaPi-IIa, the major phosphate absorbing transporter in the proximal tubule. Serum calcium and phosphate levels were mildly depleted, hypercalciuria and nephrolithiasis were observed.

Conclusions: We conclude that deficiency of renal cortical EGF increases ENaC activity and contributes to salt-sensitive hypertension. Furthermore, the disruption of the EGF-ENaC axis reported in ARPKD is linked to suppression of normal Na reabsorption, fluid accumulation in cysts and further progress of this kidney disease. Thus, EGF and other members of the EGF family are important signaling molecules in the maintaining electrolyte homeostasis in the kidney and EGF pathway abnormalities and consequent ENaC dysfunction are critical in the development of salt-sensitive hypertension and polycystic kidney diseases.

RENAL RESPONSE TO DIURETICS IN WILD TYPE AND CALBINDIN-D28K KNOCKOUT MICE

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Introduction and Aims: Renal calcium (Ca) handling is complex and modulated by multiple factors. The distal tubule is a critical nephron and considered as the fine tune of Ca transport. In this segment, Ca transport is tightly regulated through Ca transport machinery including TRPV5 and Calbindin-D28k (CBD-28k). Previous study has demonstrated that calciuria is the hallmark of Bartter Syndrome, a genetic disorder characterized by salt wasting and elevated Ca excretion. CBD-28k, a Ca transporter expressed in the kidney, has been suggested to act as a Ca channel. The exact role of CBD-28k in Ca handling remains unclear.

Methods: Both CTZ and FSM were administered to wild type mice and CBD-28k knockout mice. The 24 hr urine samples were collected to calculate urinary Ca excretion. Calcium nephrolithiasis is a complex disease with multiple pathogenetic mechanisms.
Introduction and Aims: Experimental studies in rats exposed to exogenously administered mineralocorticoids suggest the possibility of water-free storage of sodium via incorporation into glycosaminoglycans (GAG). Recently, water-free sodium-storage has been proposed for hypertensive and even healthy humans. We hypothesized that patients on dialysis exhibit a decreased capacity to excrete sodium resulting in increased tissue sodium concentrations, GAG content and expression of XYLT-1, the enzyme initiating GAG synthesis.

Methods: We studied 15 patients on dialysis undergoing renal transplantation from a live donor. Donors served as healthy controls. All patients were free of clinically detectable edema. During transplant surgery, abdominal skin, muscle and arteries were biopsied. Sodium concentration was determined by inductively coupled plasma - optical emission spectrometry after microwave digestion, semiquantitative GAG content with Alcian stain and XYLT-1 expression by real-time PCR.

Results: Adequate samples for analysis were available from 11 recipients and 2 healthy donors. Recipients and donors were comparable with respect to age (56.1 vs. 62.7 years), BSA (1.86 vs. 2.00 m²), systolic blood pressure (135 vs. 137 mmHg) and serum sodium (140 vs. 139 mmol/l). Donors tended to have a higher BMI (27.7 vs. 23.6 kg/m², p=0.04). Tissue sodium concentration was significantly higher in arteries of recipients with 3.26 ± 1.03 vs. 1.47 ± 0.86 g/kg wet weight (p=0.04). Despite clinical euvoemia, tissue sodium concentrations of both, arteries and skin, were ranging between 0.86 and 4.59 g/kg wet weight. Skin sodium concentrations of individual patients were significantly correlated with their respective sodium concentrations in muscle and arterial tissue (figure, R²=0.49, p=0.008). Blinded semiquantitative analysis of GAG staining correlated significantly with tissue sodium content (p=0.01, R²=0.483). XYLT-1 expression in muscles and arteries was also correlated with tissue sodium content (p=0.001, R²=0.624).

Conclusions: Our data confirm the observation of highly variable skin sodium concentrations in humans and extend this observation to tissue sodium concentrations in human arteries and muscles. These data support the hypothesis of water independent sodium storage via GAG synthesis in human tissues, including arteries. This mechanism may represent the link between sodium-loading and arteriolar angiotensina and deserves further study.

Methods: In order to evaluate their responses to changes in water balance, male Hyal1-/- and Hyal2-/- mice were compared to the respective wild-type (WT) mice. In the first experiment, 24h-urine collection was obtained at baseline after appropriate acclimatization, as well as after 24h of water deprivation. In the second experiment, after baseline measurements, the capacity to excrete a water load was tested on an hourly basis during the 6h following i.p. injection of 2 ml of sterile water. Urinary osmolarity as well as Na+ and K+ urinary excretion were measured. HA content was measured either by ELISA or histochemistry in kidney samples.

Results: In baseline conditions, Hyal1-/- mice, compared to the WT mice, are characterized by a lower diuresis (890 ± 79 vs 1159 ± 60 μl/24h, P<0.05) associated with a higher urine osmolarity (5015 ± 339 vs 4037 ± 304 mOsm/l, P<0.05). In contrast, Hyal2-/- mice did not present any difference regarding these parameters in comparison with the WT mice (1059 ± 61 vs 1312 ± 160 μl/24h and 3153 ± 164 vs 3357 ± 287 mOsm/l, NS). After 24h water deprivation, Hyal1-/- mice were characterized by an impaired ability to concentrate urine in comparison with WT mice (5088 ± 345 vs 5716 ± 281 mOsm/l, P<0.05), while no differences were noticed in Hyal2-/- mice. Moreover, Hyal1-/- and Hyal2-/- mice demonstrated a significant delay in the diuretic response induced by an acute water loading (Fig. 1 & 2). Nevertheless, at the end of the 6h urine collection period, the total amount of excreted water was similar in both groups. Regarding intrarenal HA in baseline conditions, both Hyal1-/- and Hyal2-/- mice present a higher HA content in comparison with WT mice. After...
24h water deprivation, HA content was significantly decreased in both WT mice. It was also the case in Hyal1-/- and Hyal2-/- mice, but HA content in Hyal2-/- mice nevertheless remained higher than in WT mice. Two hours after acute water loading, HA content tended to increase but there were no differences between KO and WT mice.

Conclusions: Taking together, our data demonstrated that the mechanism of urine concentration was impaired in Hyal1-/- and Hyal2-/- mice, thereby demonstrating the importance of intrarenal HA dynamics in renal water handling, that remains to be further investigated.