STRONG CHILDREN'S RESEARCH CENTER

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ABSTRACT

Title: Metabolic acidosis impairs clearance of UPEC-UTI

Background: Acute pyelonephritis (AN), commonly caused by vesicoureteral reflux (VUR), is a serious kidney infection in children and is often associated with metabolic acidosis. Previous studies from the laboratory have shown that metabolic acidosis induced by NH_4Cl supplementation in food impairs clearance of uropathogenic *E. coli* (UPEC-UTI) in a refluxing mouse model. NH_4Cl supplementation in H_2O has been associated with dehydration and increased aquaporin-2 (*Aqp2*) expression, which is upregulated in response to arginine vasopressin (AVP).[1,2] AVP signaling via V2R attenuates Toll-like receptor 4 (TLR4)-dependent inflammatory responses and thereby impairs UPEC clearance.[3]

Objective: To determine whether NH_4Cl supplementation in food upregulates *Aqp2* gene expression in the collecting duct (CD), as an indicator of dehydration in the mouse model.

Methods: C57Bl/6 mice (8-10 wks.) were split into three different conditions: 2% NH_4Cl supplemented food; 2% NH_4Cl supplemented food in conjunction with continuous acetazolamide (ACZ) dosage of 50 mg/kg/day via Alzet osmotic pump; and normal diet. Kidneys were harvested at 3 days, and total RNA was isolated from collecting duct segments enriched by DBA-lectin mediated magnetic sorting. Since *Cxcl12* (SDF-1) gene expression is upregulated in mice experiencing metabolic acidosis through HIF1α-dependent regulation, we examined expression of HIF1α target genes *Defb2* (β-defensin 2) and *Camp*.[4,5] Relative abundance of *Aqp2*, *Cxcl12*, *Cxcr4*, *Lcn2*, and antimicrobial peptides *Defb1*, *Defb2*, and *Camp* mRNA was quantified by qRT-PCR, with *Gapdh*, *Actb* (β-Actin), and *Cdh1* (E-Cadherin) as references.

Results: The s[HCO_3^-], urine pH, and UPEC burden/norm for normal mice were 22.2 ± 0.7, 6.8 ± 0.0, and 1; for NH_4Cl-fed mice were 15.5 ± 0.3, 5.8 ± 0.02, and 10^3-10^5; and for NH_4Cl-fed mice in conjunction with ACZ were 14.4 ± 0.4, 6.8, and 10^3, respectively. In acidotic mice, *Cxcl12* mRNA abundance was induced 4.3 ± 0.4 fold, and *Aqp2* mRNA abundance was decreased 0.5 ± 0.1 fold. Expression of the *Defb2* and *Camp* mRNA in mouse CD was 5.5×10^3 - 7.3×10^4 fold less than *Defb1*, which is known to be expressed in the CD, and was not induced by acidosis.[6]

Conclusion: Decreased *Aqp2* gene expression in acidotic mice eliminates modulation to TLR4 signaling by AVP upregulation as a possible cause of the persistent infection. Normalization of urine pH in acidotic mice with ACZ treatment did not change UPEC burden. Thus, impaired clearance of UPEC-UTI cannot be explained by an effect of urine acidification on urothelial barrier function. Since metabolic stress-associated acidosis impairs UPEC clearance, correction of acidosis may limit renal injury-associated pyelonephritis.

References:

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