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 NH₄Cl supplementation in food impairs clearance of uropathogenic E. coli (UPEC-UTI) in a refluxing mouse model. NH₄Cl supplementation in H₂O has been associated with dehydration and increased aquaporin-2 (Aqp2) expression, which is upregulated in response to arginine vasopressin (AVP). AVP signaling via V2R attenuates Toll-like receptor 4 (TLR4)-dependent inflammatory responses and thereby impairs UPEC clearance.

Objective: To determine whether NH₄Cl 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% NH₄Cl supplemented food; 2% NH₄Cl 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. 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[HCO₃⁻], urine pH, and UPEC burden/norm for normal mice were 22.2 ± 0.7, 6.8 ± 0.0, and 1; for NH₄Cl-fed mice were 15.5 ± 0.3, 5.8 ± 0.02, and 10³-10⁵; and for NH₄Cl-fed mice in conjunction with ACZ were 14.4 ± 0.4, 6.8, and 10³, 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 × 10³ - 7.3 × 10⁴ fold less than Defb1, which is known to be expressed in the CD, and was not induced by acidosis.

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:


