Aristolochic Acid-Induced Nephropathy Is Attenuated In Mice Lacking The Neutral Amino Acid Transporter B0AT1 (SLC6A19)

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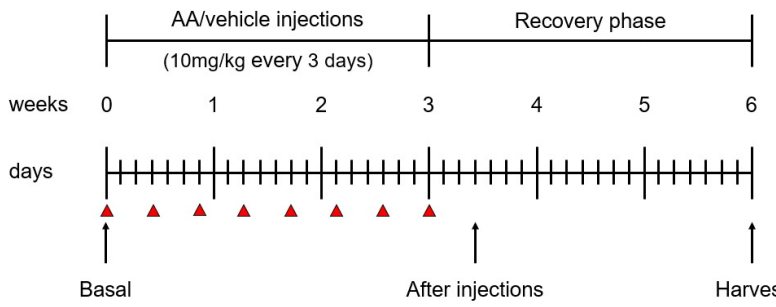
Introduction

Aristolochic acid (AA) induces acute kidney injury (AKI) and chronic kidney disease (CKD) in humans and rodents[1]. The S2 and S3 segments of the proximal tubule are primary sites of action of AA in mice and rats, respectively[2] (human data unknown). The amino acid transporter B0AT1 (SLC6a19) plays a dominant role in intestinal absorption and renal reabsorption of neutral amino acids and, in the kidney, is primarily expressed in proximal tubule S1 and S2 segments[3]. Neutral amino acids transported by B0AT1 include the branched-chain amino acids valine, isoleucine and leucine[4], the restriction of which may improve metabolic health[5]. Notably, mice lacking B0AT1 have elevated levels of FGF21 and GLP-1 and improved glycemic control[6]. Furthermore, mutations in B0AT1 have been associated with reduced serum levels of creatinine, suggesting that B0AT1 inhibition may potentially confer kidney protection[7]. In the current study, mice with whole-body knockout of B0AT1 were used to determined whether the absence of this amino acid transporter protects the kidney from injury, inflammation or fibrosis when exposed to AA.

Methods and Materials

Experiments were performed on 10- to 17-week-old female B0AT1-deficient (KO) mice and littermate B0AT1 heterozygous (HET) and wild-type (WT) mice, all on C57BL/6J background. Mice were given intraperitoneal injections of 10 mg/kg of AA or vehicle (shown by red arrowheads) every 3 days for 3 weeks followed by a 3 week recovery phase. Plasma creatinine and urinary albumin/creatinine ratios (UACR) were measured under basal conditions, 3 days after the last injection to study the active phase ("After injections"), and 3 weeks after the last injection when kidneys were harvested to study the remodeling phase ("Harvest"). Kidneys were analyzed by Masson's trichrome staining, and kidney cortices were used for quantitative RT-PCR analyses.



Statistical analysis by 2-way ANOVA, followed by Holm-Sidak method; inter, interaction; * P<0.05 vs WT # P<0.05 vs vehicle

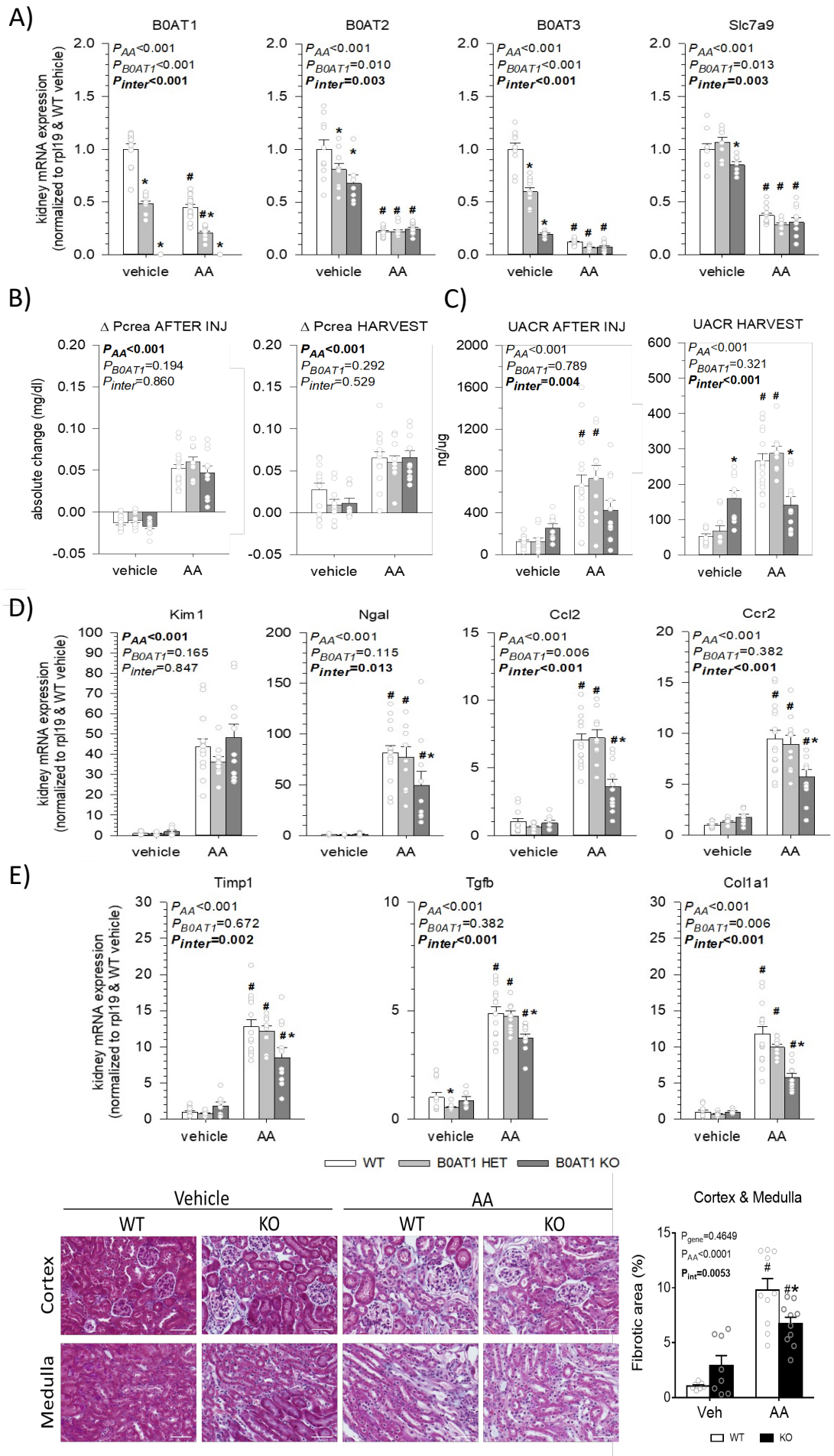
Results

A) Renal mRNA expression of B0AT1 was undetectable in KO, confirming the knockout. B0AT1 expression correlated positively with the expression of related amino acid transporters B0AT2 (Slc6a15) and B0AT3 (Slc6a18) across genotypes. In this regard, Slc6a18 and Slc6a19 are co-localized on the same chromosome. AA decreased the expression of proximal tubule transporters of neutral (B0AT1, B0AT2, B0AT3) and positively charged amino acids (Slc7a9) largely independent of genotype.

B) Changes in plasma creatinine versus basal in response to vehicle and the increase in response to AA were similar in WT and KO. **C)** UACR was higher in KO vs WT in vehicle-treated mice. AA increased UACR in WT at completion of injections and harvest, but this effect was prevented in KO, suggesting that B0AT1 promoted AA-induced albuminuria.

D) AA enhanced mRNA expression of Kim-1 independent of genotype, while increases in the expression of Ngal, Ccl2 and Ccr2 in response to AA were significantly attenuated in KO versus WT, indicating that B0AT1 promoted AA-induced injury and inflammation.

E) The AA-induced increases in renal expression of pro-fibrotic genes Timp1, Tgf-β1 and Col1a1 were likewise attenuated in KO versus WT mice. In accordance, absence of B0AT1 reduced the AA-induced upregulation of collagen staining in cortex and medulla versus WT.



References

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Conclusions

The neutral amino acid transporter B0AT1 is important for the proximal tubular physiology of albumin and glucose handling (data for glucose not shown), but plays a deleterious role in AA-induced nephropathy. These findings broaden our understanding of the role of B0AT1 and further support the therapeutic potential of inhibiting intestinal uptake or renal reabsorption of neutral amino acids to treat metabolic and renal diseases.

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