Small molecule inhibition of SLC6A19 for the treatment of chronic kidney disease

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Introduction

SLC6A19 is a Na+-dependent neutral amino acid transporter expressed on the small intestine brush border and kidney proximal tubule. Analyses of large human datasets identified a link between loss of function variants in SLC6A19 and improved renal function. In agreement with the protection from CKD observed in humans, we have demonstrated that ablation of SLC6A19 in mice protects animals from renal injury induced by the proximal tubule toxin aristolochic acid I (AAI). To investigate the pharmacological effect of SLC6A19 inhibition on AAI-nephropathy, Maze Therapeutics developed inhibitors of mouse SLC6A19 and tested them as monotherapy and in combination with the SGLT2 inhibitor, dapagliflozin.

Human genetics analysis identifies SLC6A19 as a novel target for CKD

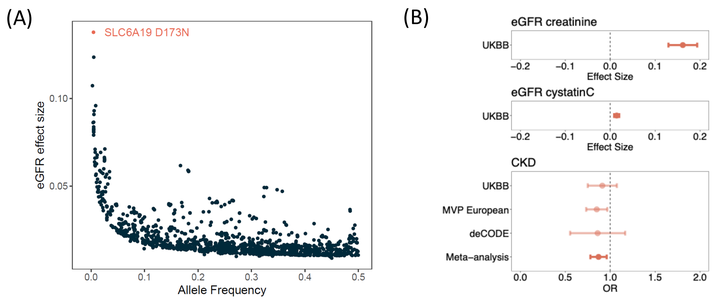


Figure 1. (A) Plot of eGFR effect sizes from single variant eGFR GWAS in UKBB highlights potential renoprotection of SLC6A19 loss of function. (B) Plot showing SLC6A19 missense variant D173N (rs121434346) on eGFR in UKBB and CKD risk across different human genetics datasets, $p < 5e^{-08}$

MZ-402 is a potent mouse SLC6A19 inhibitor

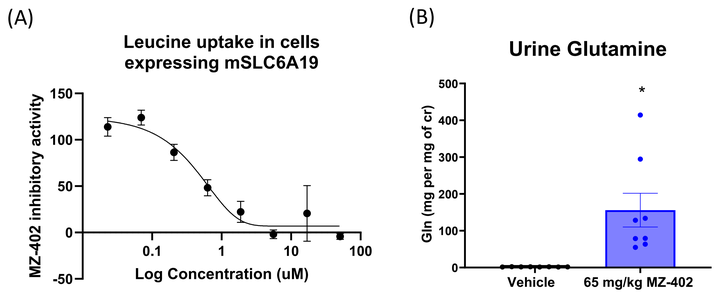


Figure 2. (A) MZ-402 inhibited leucine uptake in CHO cells overexpressing mouse SLC6A19/TMEM27. **(B)** MZ-402 or vehicle was dosed PO in C57Bl6 male mice. Urine was collected over 4 hours to measure amino acid excretion. Urine glutamine was significantly increased with MZ-402 treatment, indicating SLC6A19 engagement in vivo. * p = 0.01, two-tailed student's T-test

Aristolochic Acid I (AAI) induces nephropathy and is not a SLC6A19 substrate

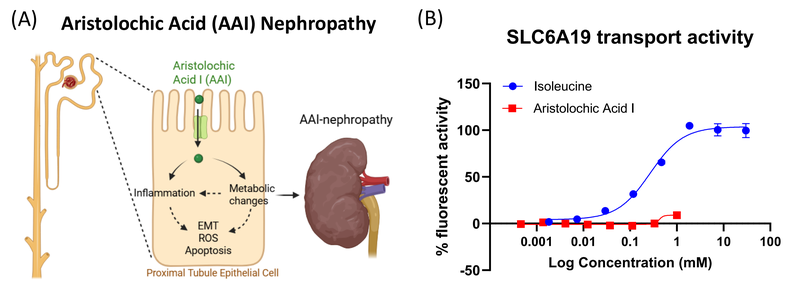


Figure 3. (A) Schematic of AAI damage: AAI is taken up in the proximal tubule epithelial cells and induces a variety of changes including inflammation and metabolic changes, resulting in apoptosis and nephropathy. **(B)** Evaluation of isoleucine and AAI transport activity in cells expressing SLC6A19/TMEM27 using a FLIPR membrane potential assay. Increasing concentrations of isoleucine (blue) resulted in increased cell membrane depolarization, indicating SLC6A19 substrate transport. Increasing concentrations of AAI (red) did not result in increased fluorescent activity, indicating that AAI is not transported by SLC6A19.

MZ-402 and dapagliflozin result in SLC6A19 and SGLT2 target engagement, respectively

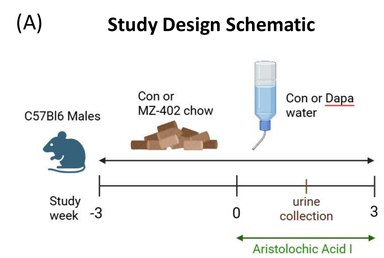
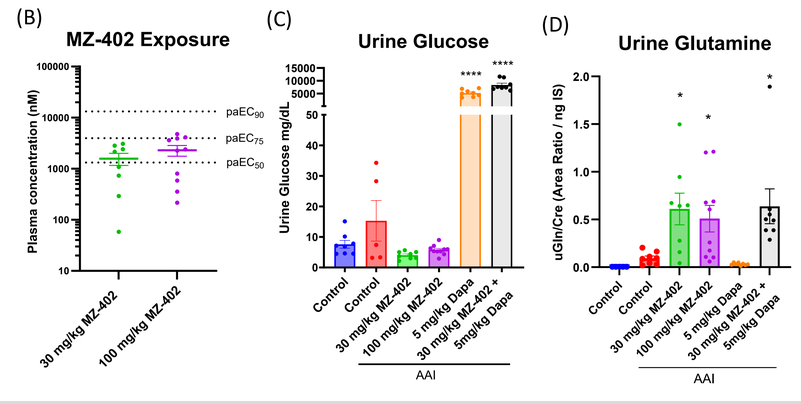


Figure 4. (A) Study design. C57Bl6 male mice were given rodent chow formulated with or without MZ-402 at 30 mg/kg/24hrs or 100 mg/kg/24hrs for 3 weeks. During this period mice were administered control (Con) or dapagliflozin at 5mg/kg/24hrs in drinking water. Mice were then treated with vehicle or AAI at 1.5 mg/kg 2x/week for 3 weeks. **(B)** MZ-402 plasma exposure of between protein-adjusted (pa) EC_{50} and EC_{75} (based on *in vitro* Leu uptake assay) was achieved by MZ-402 administration through rodent chow formulation. **(C)** Urine glucose demonstrating SGLT2 target engagement with dapagliflozin treatment. MZ-402 did not induce urine glucose excretion, demonstrating that it does not block SGLT2. **(D)** Urine glutamine demonstrating SLC6A19 target engagement with MZ-402. * p<0.05, **** p<0.0001 One way ANOVA comparison with non-AAI treated control.



Inhibition of SLC6A19 protects against AAI-induced kidney injury through a differentiated mechanism to SGLT2i

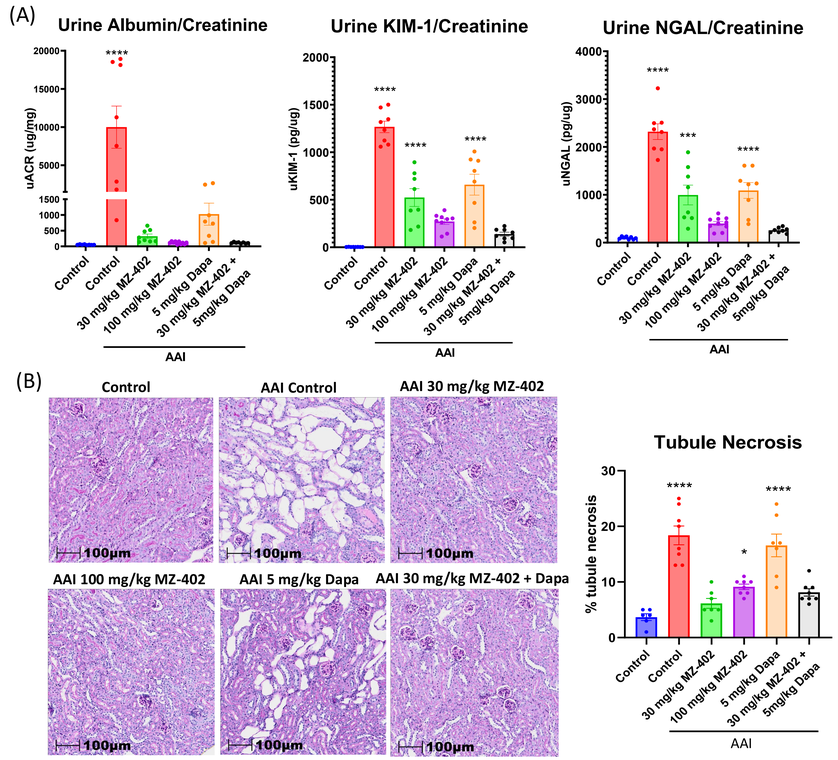


Figure 5. (A) Analysis of urine kidney injury biomarkers at week 1.5. MZ-402 reduced AAI-induced increases in uACR, uKIM1, and uNGAL. These effects were augmented with dapagliflozin treatment. **(B)** H&E staining and analysis at week 3 demonstrating kidney tubule atrophy in the AAI model that is reduced by SLC6A19 inhibition. Tubule necrosis analyzed using HALO and plotted as percent of kidney section. *** p<0.001, **** p<0.0001 One way ANOVA comparison with non-AAI treated control

Conclusions and Acknowledgments

- MZ-402 is a potent inhibitor of mouse SLC6A19
- SLC6A19 inhibition protects against AAI-induced kidney injury and in combination with the SGLT2 inhibitor, dapagliflozin, further enhances its protective effects
- These data provide a strong rationale for investigation of SLC6A19 inhibition as a potential therapeutic approach for CKD. An investigational small molecule inhibitor of SLC6A19, MZE782, is currently being evaluated as a potential therapy for CKD and phenylketonuria (PKU).

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