Impact of Enzymatic Degradation of Plasma Cystine in a Mouse Model of Cystinuria Under Dehydration Challenge

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Abstract # TH-PO815

Introduction

Cystinuria is an autosomal recessive genetic disease caused by mutations in the SLC3A1 and/or SLC7A9 dibasic amino acid transporter genes. Disruption of transporter function leads to increased urine cystine concentrations that exceed the limit of solubility resulting in cystine precipitation and formation of upper tract urinary calculi, with recurrent stone passages and/or need for multiple procedural interventions. Impact to patients include:

- Episodes of severe abdominal pain
- Obstructive syndromes like hydronephrosis
- Hematuria and infective syndromes like pyelonephritis
- Chronic pain (opioid addiction risk)
- Hypertension, CKD & reduced life expectancy

Current disease management includes:

- Adequate hydration to substantially increase urine output to maintain urine [cystine] <250mg/L
- Diet modification to reduce cystine intake
- Alkalization of the urine to improve urine cystine solubility
- Thiol binding drugs to convert cystine to a more soluble form

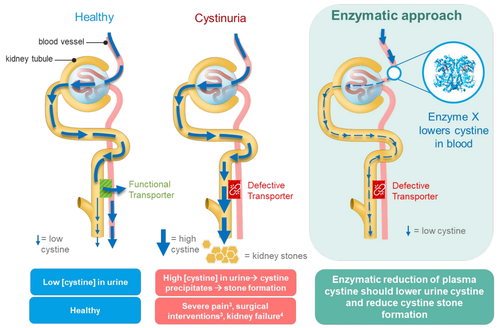
These approaches are commonly inadequate at reaching target cystine urine concentration owing to poor compliance, limited effectiveness due to poor tolerability to therapies or impact of quality of life. Examples of the latter include nocturia and occupational compliance difficulties[1,2].

Case Histories

A young man with no history of medical problems joins the armed forces of the United States of America. He passes the routine screening and physical examinations, and is deployed to the Middle East. He serves intermittently in the hot and dry environment. He initially reports no health problems. After 18 months:

- He experiences gross hematuria
- Screening examination with renal ultrasonography reveals bilateral large renal stones
- He is discharged from the armed forces and returns to the U.S. mainland
- Referral is made to one of the authors, who obtains computed tomography as part of the evaluation
- Serum creatinine is elevated, and the estimated glomerular filtration rate is 28 mL/min/1.73m^2
- He undergoes staged bilateral percutaneous nephrolithotomy
- Stone analysis reveals cystine calculi. There is no family history of cystinuria, urinary calculi or any urological or nephrological disease
- Despite aggressive management, he develops end-stage renal disease (ESRD)

Therapeutic Rationale



- With the AEB5100 program, we are developing a highly novel approach for the treatment of cystinuria patients that works by reducing cystine delivery into the kidney tubules through reduction of plasma cystine concentration
- The candidate molecule is a modified human enzyme with novel activity for cystine
- Lowering cystine in the blood is expected to:
  a) reduce the cystine load filtered to the kidney to a concentration below the precipitation point
  b) prevent stone formation
  c) reduce the excessive urine output which is critical to the effectiveness of current therapy

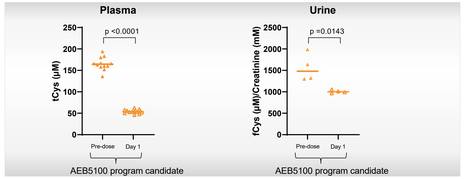
Methods

We used a murine model of cystinuria (Slc3a1-/-) that develops stones between 4 & 7 weeks of age[5] and either gave mice *ad libitum* access to full drinking water, or rationed water to 65% to mimic the challenge for patients with cystinuria to comply with high fluid intake regimens[1,2]. During water rationing, mice were untreated or administered either PBS or the AEB5100 program candidate enzyme. The concentration of total bound and unbound cysteine and reduced cystine (total cystine, tCys) in plasma was measured before and after dosing. The concentration of unbound cysteine and reduced cystine (free cystine, fCys) in urine was monitored at specific timepoints prior to and after treatment. Kidneys and bladders were resected, weighed and analyzed by microcomputed tomography (μCT) to determine the total number and volume of cystine stones in each kidney and bladder. Stats: Unpaired t-test and Mann-Whitney test.

Results

A single dose of the AEB5100 program candidate in adult (9-11.2 month old) female Slc3a1 -/- mice resulted in statistically significant reduction of plasma cystine compared to pre-dose baseline, and this reduction in plasma cystine translated to a reduction in urine cystine concentration (Fig 1).

Fig 1. Plasma and urine PD in adult cystinuria mice

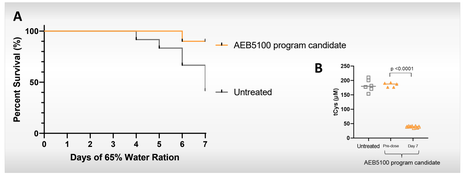


Plasma cystine (tCys) and urine cystine (fCys) concentrations in adult female Slc3a1 -/- mice (with *ad libitum* access to drinking water) before and after a single dose (IP at 50mg/kg) of the AEB5100 program candidate

Water rationing for 1 week resulted in morbidity of adult (9.5-11.5 month old) male Slc3a1 -/- mice with 41.67% survival of untreated Slc3a1 -/- mice, while dosing with the AEB5100 program candidate enzyme, during water rationing, resulted in 90% survival (Fig 2A).

Plasma collections were performed before 65% water rationing to determine the baseline cystine plasma concentration (Fig 2B). IP administration of the AEB5100 program candidate at 50 mg/kg QOD for 1 week during water rationing resulted in ~5-fold statistically significant reduction of cystine in plasma compared to baseline (Fig 2B).

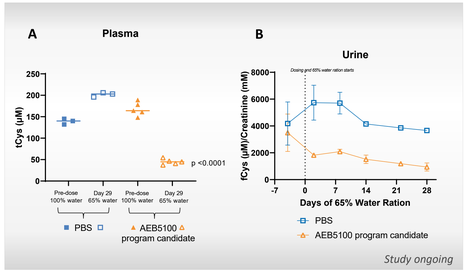
Fig 2. Survival of cystinuria mice on water ration



Survival (A) and cystine plasma concentration (B) of adult male Slc3a1 -/- mice on 65% water ration that were either untreated or dosed with the AEB5100 program candidate

Dosing of the AEB5100 program candidate in young (5-6 weeks old) male Slc3a1 -/- mice for 4 weeks during water rationing resulted in statistically significant reduction of cystine plasma concentration compared to pre-dose baseline and compared to mice dosed with PBS (Fig 3A). This reduction in plasma cystine translated to a reduction in the concentration of cystine in urine over time (Fig 3B).

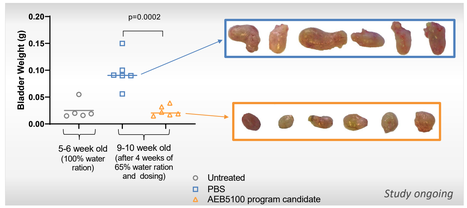
Fig 3. Plasma and urine PD in young cystinuria mice



Plasma cystine (A) and urine cystine (B) concentrations of young male Slc3a1 -/- mice on 65% water ration dosed with either PBS or the AEB5100 program candidate for 4 weeks

At terminal collection, the bladder weights of male Slc3a1 -/- mice that were dosed with the AEB5100 program candidate were statistically significantly less than those of Slc3a1 -/- mice dosed with PBS, and comparable to those of untreated control Slc3a1-/- mice of 5-6 weeks of age that were given *ad libitum* access to drinking water and were used as control to measure bladder weights at the age of dosing and water ration initiation.

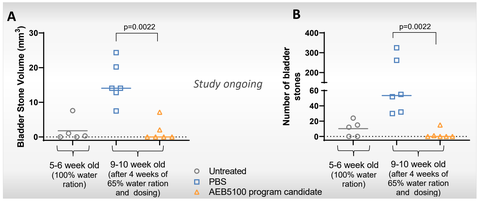
Fig 4. Bladder weights of young cystinuria mice



Bladder weights of young male Slc3a1 -/- mice that were either untreated and given *ad libitum* access to drinking water, or were dosed with PBS or the AEB5100 program candidate for 4 weeks during 65% water ration

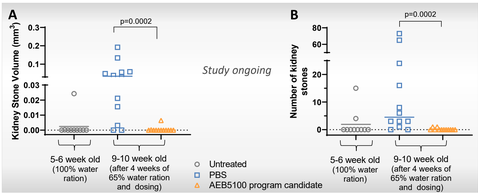
The number and total volume of cystine stone per kidney and per bladder of male Slc3a1 -/- mice that were dosed with the AEB5100 program candidate were statistically significant smaller than those of mice dosed with PBS, and comparable to those of untreated control Slc3a1-/- mice of 5-6 weeks of age (age of dose initiation) that were given *ad libitum* access to drinking water. These data indicate that the AEB5100 program candidate prevents stone formation and growth in the Slc3a1-/- model, even under conditions of limited hydration.

Fig 5. Number and volume of stones per bladder



Volume (A) and number (B) of bladder cystine stones of young male Slc3a1 -/- mice that were either untreated and given *ad libitum* access to drinking water, or were dosed with PBS or the AEB5100 program candidate for 4 weeks during 65% water ration

Fig 6. Number and volume of stones per kidney



Volume (A) and number (B) of bladder cystine stones of young male Slc3a1 -/- mice that were either untreated and given *ad libitum* access to drinking water, or were dosed with PBS or the AEB5100 program candidate for 4 weeks during 65% water ration

Conclusion

The AEB5100 program candidate when administered to a cystinuria mouse model under dehydration challenge:

- reduces the cystine urine concentration, by lowering cystine in the blood
- prevents stone formation and growth in the kidneys and bladder

This data support further development of the AEB5100 program candidate as a novel approach for the treatment of cystinuria patients who are:

- unresponsive to current treatment approaches,
- intolerant to current therapies or
- facing quality of life factors that impact the ability to comply with current treatment approaches

References 1. Rozanski et al, Mil Med (2005)) 2. Soucie et al, JM Kidney Int (1994) 3. Biyani and Cartledge EAU-EBU Update Series 4 (2006) 175-183 4. Leslie and Nazzal Renal Calculi (Cystinuria, Cystine Stones) (2018) Reference 5. Zee et al, Nature Medicine (2017)

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Disclosures Giulia Agnello, Jason Wiggins, Silvia Ferrati, Anthony Quinn, and Scott Rowlinson are employees of and have an equity interest in Aeglea BioTherapeutics, Inc.