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Consumption of Uric Acid in the Gastrointestinal Tract with Engineered *E. coli* Nissle as a Potential Treatment for Gout

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INTRODUCTION

Elevated circulating levels of uric acid (UA) are associated with an increased risk of developing gout and other chronic diseases such as chronic kidney disease (CKD), type 2 diabetes (T2D), and cardiovascular disease (CVD). Despite advancements in treatment options, gout is often poorly managed[1]. In healthy individuals, about 70% of circulating UA is metabolized and excreted by the kidneys, while the remaining 30% is mainly excreted with the feces or further catabolized by the gut microbiota. Not surprisingly, circulating UA is commonly elevated in subjects with impaired kidney function. Furthermore, studies have found that gut microbiota dysbiosis can contribute to the reduced capacity for host UA degradation in gout patients. In light of these findings, we engineered a therapeutic probiotic, SYN-GOUT, to degrade UA within the GI tract for the treatment of gout.

We have successfully demonstrated the utility of using engineered probiotics for metabolic diseases. For example, probiotic bacterium *E. coli* Nissle (EcN) 1917 was engineered to treat phenylketonuria (PKU)[2,3]. The PKU strain is currently being evaluated in clinical trials.

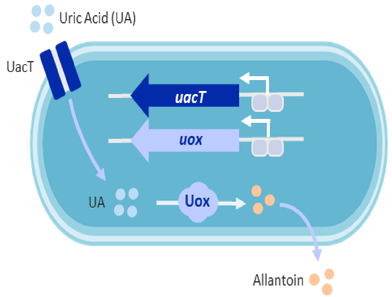

Figure 1. Schematic showing engineered components of SYN-GOUT strain of EcN capable of importing and degrading UA to allantoin. The *uox* gene originates from *Chimaeribacter californicus* and the *uacT* gene from *Edwardsiella tarda*.

METHODS

- SYN-GOUT is a prototype strain with engineered UA degradation components that are plasmid-based
- SYNB2081is a clinic-ready strain with engineered pathways integrated into the chromosome of E. coli Nissle and all FDA-required biocontainment pieces in place
- SYN-GOUT *in vitro* activity was assessed in minimal culture media
- Male C57/B6 mice were used for the enterorecirculation experiments
- The *in vivo* activity of the candidate strain SYNB2081 was determined in non-human primates (Charles River Laboratories, Shrewsbury, MA)

RESULTS

SYN-GOUT consumes UA *in vitro*, and its activity remains high under low O_2 conditions

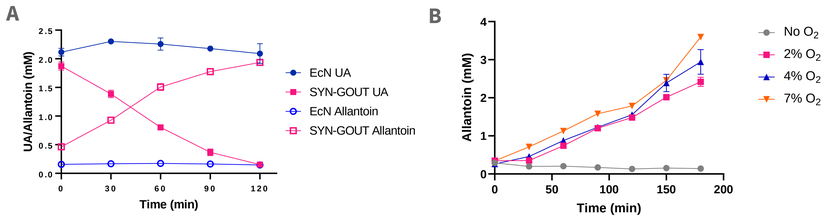

Figure 2. **A**. Consumption of uric acid and production of allantoin *in vitro*. 1E9 cells were added to minimal media containing 1mM UA and 0.5% glucose and incubated at 37˚C for 2hrs. **B**. Strain activity as measured by production of allantoin under various dissolved oxygen (DO) conditions. 1E9 cells were added to minimal media containing 5mM UA and 0.5% glucose and incubated at 37˚C over 3hrs.

The circulating pool of UA is accessible from within the gut

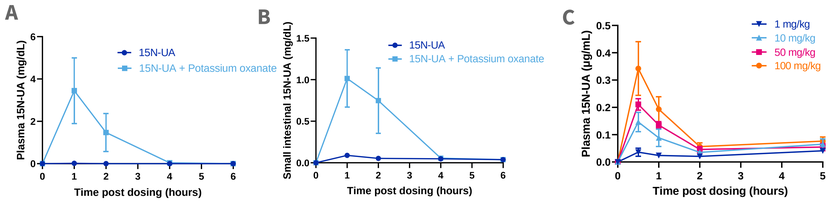

Figure 3. *In vivo* demonstration of an enterorecirculation loop for UA. In **A** & **B**, C57B/6 mice were given labeled UA ($1,3\text{-}^{15}N_2$) *via* intraperitoneal route in the presence or absence of an uricase inhibitor, potassium oxonate. Accumulation of labeled UA was detected in both plasma (**A**) and small intestinal contents (**B**) when uricase activity was inhibited. In (**C**), C57/B6 mice were given an increasing amount of labeled UA *via* oral gavage, and the appearance of labeled UA in plasma was measured. N = 8 animals/group.

SYNB2081 lowers plasma and urinary levels of UA, with a concomitant increase in allantoin production in nonhuman primates

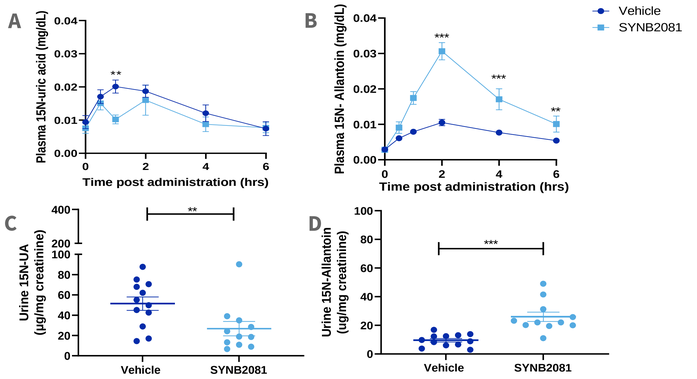

Figure 4. Demonstration of SYNB2081 activities in non-human primates. NHPs were dosed with vehicle or 1E12 cells in the presence of labeled uric acid. Plasma (**A**) and urinary (**C**) levels of labeled UA were significantly reduced in SYNB2081 (*vs* vehicle) group. Conversely, plasma (B) and urinary (D) levels of allantoin were elevated in SYNB2081 (*vs* vehicle) group. $^{**}p < 0.01$, $^{***}p < 0.001$, n = 6 animals/group.

CONCLUSIONS

- SYN-GOUT consumed UA *in vitro* , and was active under hypoxic conditions.
- SYNB2081 was safe and well-tolerated in NHPs; furthermore, it significantly lowered plasma and urinary levels of UA, indicative of its robust activity *in vivo*.
- SYNB2081 could be an effective alternative for the treatment of gout, especially in individuals with compromised kidney function.

REFERENCES

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3. Isabella et al., Nat Biotechnol 2018 Oct;36(9):857-864.

Disclosures

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