Randomized, Placebo-Controlled Study of Lyophilized Formulation of SYNB1618 in Healthy Adult Volunteers

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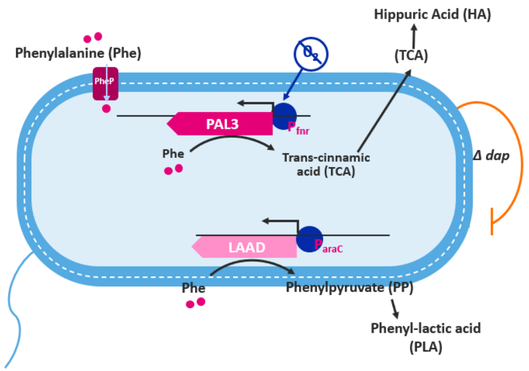
Background

Dietary control of phenylalanine (Phe) intake is a primary method of management of phenylketonuria (PKU), but due to its highly restrictive nature is difficult to follow. Despite recommendations supporting life-long control of Phe levels and existing treatments, some children and most adults continue to have Phe levels above the recommended range, putting them at risk of poor cognitive outcomes. SYNB1618, a live, modified strain of the probiotic bacterium E. coli Nissle was engineered to consume phenylalanine (Phe) in the gastrointestinal tract (GI) as a novel treatment approach. The safety, tolerability and pharmacodynamics (PD) of multiple doses of a solid oral formulation of SYNB1618 were evaluated in healthy volunteers (HV).

Methods

SYNB1618 is a non-colonizing strain genetically engineered to contain genes encoding phenylalanine ammonia lyase (PAL), which converts Phe to *trans*-cinnamic acid (TCA), and ammonia. TCA is further converted to hippuric acid (HA) by the host and excreted in urine. A second Phe degradation pathway in the strain is through the enzyme L- amino acid deaminase (LAAD), which converts Phe to phenylpyruvate. Phenylpyruvate is further degraded by multiple pathways in the host, including conversion to phenyllactate, which is excreted in urine. For biocontainment the strain is a diaminopimelate (dap) auxotroph. A schematic of the strain design is shown below.



Study Design

Healthy volunteers were enrolled in a *multiple ascending dose (MAD),* randomized, double-blind, placebo controlled study. They received ascending doses of SYNB1618 TID with or without a proton-pump inhibitor (PPI) (esomeprazole 40mg QD) or prophylactic ondansetron for up to 3 days. Additionally, in Part 2 SYNB1618 was dosed open-label for up to 7 days at increasing doses. To allow for assessment of strain-specific biomarker production a ***tracer study*** was conducted at baseline and on the last day of dosing. Subjects received 15/mg/kg of D5-Phe and a 20 g protein load (meal replacement shake) with blood and urine collection over 6hrs. The ***biomarker study*** measured production (TCA) in the fasted state with blood and urine collection over 6hrs after an overnight fast following a single dose of SYNB1618.

Study Outcomes

Primary objective: safety and tolerability. Secondary objectives: SYNB1618 microbial fecal kinetics by strain-specific qPCR, tolerability using a dose-ramp or a prophylactic ondansetron. Exploratory endpoints: Pharmacodynamic (PD) effects of SYNB1618 on strain-specific biomarkers TCA and HA.

Results

Study Population

A total of 88 healthy volunteers were enrolled; 72 subjects in over 9 MAD cohorts and 16 subjects in Part 2 in 2 dose-escalation cohorts; majority were Caucasian (86%), age range 18-63 y, 39% female. The study dose ranged from 1 x 10^{11} to 4 x 10^{12} live cells TID.

Safety

The solid oral formulation of SYNB1618 was well tolerated. The MTD was 2 × 10^{12} live cells. The most commonly occurring TEAEs were GI-related events and headache; the majority of these events were mild or moderate. Nausea and vomiting were the dose-limiting symptoms. Adding a dose-ramp in Part 2 Cohort 2 improved the tolerability of the 2 × 10^{12} dose and decreased the incidence of AEs. Prophylactic use of ondansetron did not prevent nausea and vomiting. No deaths or SAEs occurred during the study. One subject withdrew from the study due to vomiting. A temporary dose-related increase in CRP was observed in some subjects; the clinical significance of this finding is unclear. All subjects cleared SYNB1618 from their stool within 7 days after the last dose.

Tracer Study: PD effects

Dose-dependent reductions in D5-Phe AUC were observed in subjects administered SYNB1618 compared with placebo subjects after the protein load in the tracer study, but not in unlabeled Phe AUC. A corresponding increase in strain-specific biomarkers D5-TCA and D5-HA was observed clearly demonstrating strain consumption of Phe within the GI tract. Figure 1.

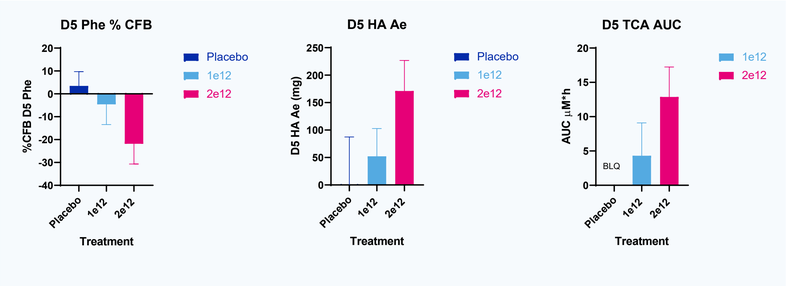


Figure 1. A dose-related decrease in plasma D5-Phe AUC0-6h with corresponding increases plasma D5-TCA AUC0-6h and urine D5-HA amount excreted were observed in SYNB1618 treated groups compared to placebo after a protein load (20g of intact protein and 15 mg/kg D5-Phe). (Data represent mean and 90% CI) [CFB=change from placebo; Ae=amount excreted]

The maximum plasma TCA concentration was observed at 90-120 minutes postdose, and the biomarker was detectable for up to 5 hours suggesting activity primarily in the stomach and small intestine. Figure 2.

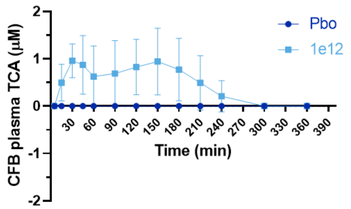


Figure 2. Plasma TCA concentration measured during the tracer study; data shown for the 1 x 1012 dose. Other dose levels showed a similar pattern. [mean change from baseline (CFB) ±SD]

Activity of SYNB1618 without dietary protein load

Biomarker Study (fasted state)

In a placebo-controlled cohort after an overnight fast on Day 1, subjects were given 2 × 10^{12} live cells of SYNB1618. They remained fasted until blood and urine sample collection was completed after 6 hrs. The production of TCA in the fasted state was similar in magnitude to the production after a protein load in the tracer study on Day 2 in the SYNB1618 treated group. *Figure 3* No TCA production was observed in the placebo group as expected.

Similarly, in an uncontrolled SYNB1618 cohort in Part 2 dosed with 2 × 10^{12} live cells of SYNB1618 after an overnight fast, plasma Phe level measured 2hrs later tended to decrease slightly in the fasted state compared to an increase after a 20g protein load. *Figure 4.* These data indicate presence of resident Phe in the gut even in the fasted state enabling strain activity independent of dietary Phe intake.

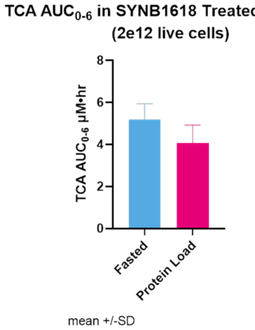


Figure 3. Plasma TCA AUC0-6h was similar in the fasted state compared to production after a 20g protein load.

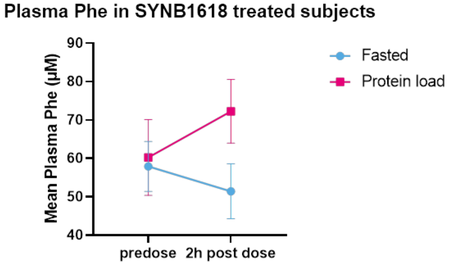


Figure 4. Plasma Phe measured after an overnight fast (predose) and 2 hrs after a single 2x10^{12} dose of SYNB1618 in subjects who on two separate days received a protein shake (red) or remained fasted (blue).

Conclusions

- The solid oral formulation of SYNB1618 was well tolerated and metabolically active in the human GI tract.
- SYNB1618 reduced the increase of plasma D5-Phe in the tracer study in a dose-dependent manner in healthy volunteers.
- SYNB1618 demonstrated evidence of activity even without protein intake (fasted state), suggesting resident Phe in the GI tract.
- These data support the further clinical development of this live biotherapeutic therapy for the treatment of PKU. A Phase 2 proof-of-concept study SynPheny-1 [NCT04534842] is ongoing.