Abstract
Phenylketonuria (PKU) is an inherited metabolic disorder resulting from the inability of the liver to break down Phenylalanine (Phe) leading to the dysregulation of metabolism in the brain and neurotoxicity. To develop new alternative approaches to a strict low-protein diet we have designed a genetically engineered strain of Escherichia coli Bacteria that can metabolize Phe in the mammalian gut. The engineered strain, SYN1618, has been designed to convert Phe into trans-cinnamate (TCA) with the phenylalanine amino transferase (PAL) enzyme or to phenylpyruvate (PP) via the L-phenylalanine amiono transferase (LAAT) enzyme.

Previously we have shown that oral dosing of SYN1618 can mediate dose dependent decreases in circulating Phe levels and increases in TCA (or its downstream metabolite Hippurate, HA) or PP in primate models or in healthy human volunteers. Initial trials have utilized a frozen liquid cell suspension, however, methods to develop a solid dosage form are desirable to improve stability, enable outpatient studies, and improve ease of use for patients. To that end, we have developed a process for lyophilization of SYN1618 cells which does not impact cell morphology or significantly compromise viability or bioactivity. Lyophilized cells retain the ability to consume Phe and produce TCA or PP in vitro or produce TCA in an in vivo, intestinal simulation model. Furthermore, the lyophilized SYN1618 demonstrated similar HA production to a liquid cell suspension in both a mouse model of PKU and in healthy non-human primates. We conclude that lyophilization of SYN1618 does not result in significant loss of activity and there is a dearth path forward for a solid oral formulation in the development of SYN1618 for the potential treatment of PKU.

METHODS

RESULTS

Figure 2. Process to develop a solid SYN1618 formulation

Figure 3. Morphology and viability of solid formulation a) Electron microscopy

Figure 4. Activity of Lyophilized SYN1618 cells in vitro in media containing Phe (a), or in a in vitro gut simulation system (IVS) mimicking oxygen and pH conditions of the human GI tract (b).

Figure 5. In vitro activity of solid formulation in a PKU Mouse model and in NHPs a) Mice were administered 4.1e10 live cells over 3 doses which is the equivalent of 1.3e10, 2.1e10, and 1.4e9 CFU for ED0, frozen liquid and lyophilized formulations respectively. b) 86% of NHPs without Phe treatment and 71% of NHPs with Phe treatment were shown to have significant levels of DAP (1.34e10) or HA (1.29e10) in the gut compared to untreated NHPs which had negligible levels of either. The equivalent dose for human therapy would be less than 1.0e9 CFU.

Figure 6. Batch to batch consistency of solid process. Three independent batches were evaluated for viability, a), activity in vitro, b), or activity in vivo c) in a WT mouse

Figure 7. Analytical characterization of batches made with new solid process compared to previous frozen liquid batch

Figure 8. Stability of solid SYNB1618 formulation either at 2-8 degrees or room temperature.

CONCLUSIONS

Figure 9. Design of SYN1618 strain and activity in healthy human volunteers. a) The genome of E. coli Nissle has been modified to include additional copies of the high affinity Phe transporter (PheT) to increase Phe uptake, copies of the PAL enzyme to covert Phe to trans-cinnamate (TCA) and the LAAD enzyme to covert Phe to Phenylpyruvate. The dapA gene has been deleted to make the strain a diaminopimelate (DAP) auxotroph which limits replication in vivo and acts as a biocontainment strategy without exogenous DAP present. b) Overview of SYN1618 Phase I/2a studies in healthy volunteers and PKU patients. c) Dosing paradigm including use of OS-Phe probe to monitor SYN1618 activity. d) Plasma TCA levels following a single dose of SYNB1618 in healthy volunteers. e) Urinary HA and OS-HA levels following multiple doses of SYNB1618 in healthy volunteers.

- We have developed a process for a solid formulation of SYNB1618 with minimal impact on cell viability or activity.
- Lyophilized SYNB1618 is similarly active to frozen liquid in consumption of Phe or production of TCA/HA in vitro and in vivo.
- Process robustness has shown batch to batch reproducibility in cell viability and activity at the 30 % scale.
- Lyophilized SYNB1618 is stable for 50 days at 2-8 C and 10 days at room temperature.
- The new solid process is expected to have improved quality attributes including less free protein and reduced viscosity.
- A solid oral SYNB1618 formulation with improved stability and convenience has potential as a new therapy for managing blood Phe levels in PKU patients.