Assessing the Potential of Enzymes as Inhaled Therapeutics for Inborn Errors of Metabolism

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BACKGROUND

Classical Homocystinuria (HCU) is a genetic disorder caused by mutations in the enzyme cystathionine beta-synthase (CBS). When CBS is missing or not functioning properly, homocysteine (Hcy), and its precursor methionine (Met), builds up in the blood and body. Excess Hcy can cause symptoms to manifest in the ocular, vascular, skeletal, and central nervous systems, which can become life threatening without treatment. The standard therapy for HCU is a strict, lifelong Met-restricted diet, which is challenging to maintain and may not prevent all symptoms. Supplementation of vitamins (B6, B12, folate), as well as the medication Betaine, may provide additional benefits for some patients. Currently no other FDA-approved therapies exist, thus the need for new therapeutic options is recognized.

STUDY OBJECTIVE

In this study, we present a new treatment approach: an inhaled enzyme that would break down toxic metabolites in the lungs, thus reducing levels in the blood to improve disease symptoms.

LIST OF ABBREVIATIONS

CBS – cystathionine beta-synthase enzyme
HCU – Homocystinuria
Hcy – homocysteine
IEM – inborn error of metabolism
IP – intraperitoneal (delivery to the body cavity)
IT – intratracheal (delivery to the lung)
Met – methionine
PAH – phenylalanine hydroxylase enzyme
PAL – phenylalanine ammonia lyase enzyme
Phe – phenylalanine
PKU – Phenylketonuria
RDPAL_9 – engineered PAL enzyme
SEM – standard error of the mean
SQ – subcutaneous (delivery under the skin)
WT – wildtype (natural or unmodified)

THE TECHNOLOGY

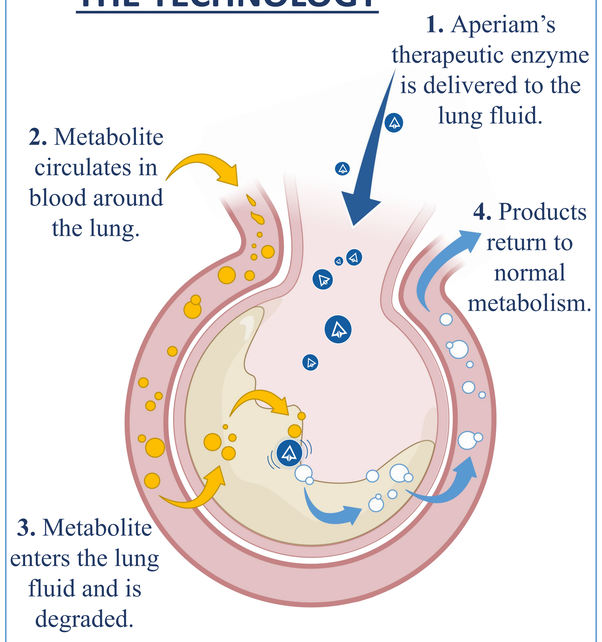


Figure 1: Lung Alveoli with Exposure to Metabolites in Circulating Blood

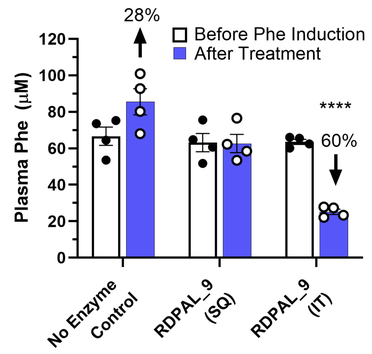
METHODS

In order to test this novel hypothesis to treat inborn errors of metabolism (IEM), we used Phenylketonuria (PKU) as a proof-of-concept case study. We started with the bacterial enzyme PAL (phenylalanine ammonia lyase from *Anabaena variabilis*) that is known to break down phenylalanine (Phe) safely, and is the same active enzyme as Palynziq® and others in clinical development. Using protein engineering techniques, we made PAL more stable for lung delivery. We then tested this engineered enzyme, termed RDPAL_9, in two mouse models: a genetic PKU disease model, and a healthy mouse model in which we artificially increased blood Phe levels. RDPAL_9 was dosed either by delivering the enzyme directly to the lungs (intratracheal, IT) or under the skin (subcutaneous, SQ). We then measured the Phe levels in the blood at scheduled time points throughout the studies.

RESULTS IN MICE

Healthy female mice were provided with Phe + a PAH inhibitor to artificially raise blood Phe, which increased levels ~28%. RDPAL_9 was then dosed either by SQ or IT delivery. SQ was able to reduce blood Phe levels to untreated baseline, while IT delivery significantly reduced blood Phe levels by 60%.

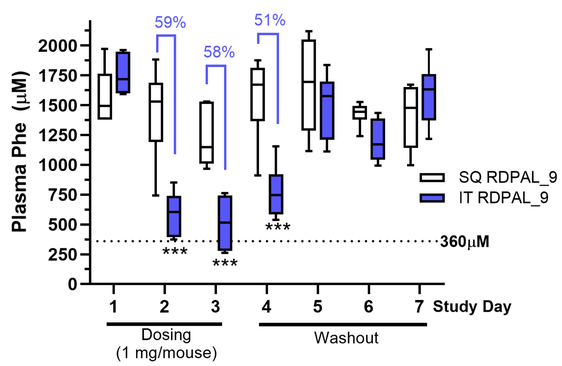
Figure 2: RDPAL_9 reduced plasma Phe in an induced mouse model of PKU



Legend: Healthy mice (n=4/group) were given daily pCP/Phe (IP) to artificially raise plasma Phe, and then either vehicle or 1 mg RDPAL_9 (3 doses in 24 hours) delivered either SQ or IT. Data shown as Mean ± SEM. Plasma Phe concentrations at Day 2 (white bars, before induction) and Day 4 (blue bars, after treatment) were compared by unpaired t-test. **** p<0.0001. Directional black arrows indicate percent increase or decrease compared to Day 2.

In PKU mice, IT delivery of RDPAL_9 reduced Phe levels by at least 51%, bringing them down to a level similar to recommended clinical guidelines. The effect persisted for two days after treatment had stopped. SQ delivery did not lower blood Phe significantly.

Figure 3: IT delivered RDPAL_9 significantly reduced plasma Phe in a PKU mouse model



Legend: PKU (PAHenu2) mice (n=6/group) were treated twice daily with 1 mg/mouse RDPAL_9, delivered IT (blue boxes) or SQ (white boxes). Animals were dosed on Days 1, 2 and 3. Blood was collected daily immediately before dosing for Phe quantification. Data is shown as the Mean ± Min/Max, with multiple unpaired t-tests comparing treatments. *** p<0.001. Percent change in Phe is noted for statistically significant days (Days 2, 3, 4) in blue text. The clinical treatment guideline threshold for PKU (360 µM) is represented by a dotted line.

ENHANCED STABILITY RESULTS

PAL was engineered to be more stable to the lung environment. Variants were screened for temperature stability, and enzyme activity was also tested at the appropriate pH for lung fluid. Engineered RDPAL_9 was more stable at high temperatures when compared to the WT PAL enzyme. RDPAL_9 was also more active than WT after being turned into a mist for inhalation (i.e. nebulized). This is especially important for IEM disorders, since infants and young children are a critical population for treatment.

CONCLUSIONS

- Engineered PAL delivered to the lungs significantly reduced Phe levels in animal models of PKU compared to SQ delivery
- The engineered enzyme displayed improved stability and activity vs the WT enzyme
- The lungs provide less risk of immune reactions compared to injectable therapies, which suggests this approach could be a safe and effective treatment for all patients, regardless of their age or specific mutation
- Importantly, these data demonstrated that the lung can be transformed into a tunable therapeutic organ capable of metabolizing a circulating toxin
- Taken together, this may translate to broader opportunities towards the development of inhaled treatments for other metabolic disorders, such as HCU

QUESTIONS?

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