One-Year Progress Report to National Tay-Sachs & Allied Diseases Association

BioStrategies LC

Lectin-assisted transnasal delivery of corrective enzyme for GM1 gangliosidosis

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Executive Summary:

Genetic deficiencies in the lysosomal enzyme β-galactosidase (β-gal) lead to the neurodegenerative disease GM1 gangliosidosis. Traditional enzyme replacement therapy (ERT) approaches are ineffective because they fail to deliver sufficient enzyme to the brain. Our goal in this research, is to determine if the plant RTB lectin (sugar-binding protein) can help to carry β-gal across nasal mucosal surfaces and increase access to the brain using the mouse model of GM1. Fusion proteins linking β-gal with the RTB lectin will be produced in a plant-based protein production system and the purified protein will be administered trans-nasally to *β-gal*$^{-/-}$ mice to determine if active enzyme reaches the cells of the brain.

The *specific objectives* of this research and proposed timeline of research milestones

1. Produce, purify, and characterize β-gal:lectin test material required to support mouse studies.

   6-Month Milestone: production, purification, and qualification (enzymatic activity, lectin binding, and cell uptake) of 1-2 mg of β-gal:RTB product to support first mouse trial.

2. Assess β-galactosidase activity in brains of *β-gal-/-* mice following a single nasal administration of β-gal:RTB.

   1-Year Milestone: β-gal activity data from initial mouse trial providing evidence of transnasal delivery of β-gal:RTB to CNS or visceral sites

3. Analyze impacts on brain GM1 levels and disease phenotype in *β-gal-/-* mice following longer-term β-gal:RTB transnasal administration

   1.5-Year Milestone: *in vivo* data documenting CNS delivery of bioactive β-gal and evidence of disease correction.

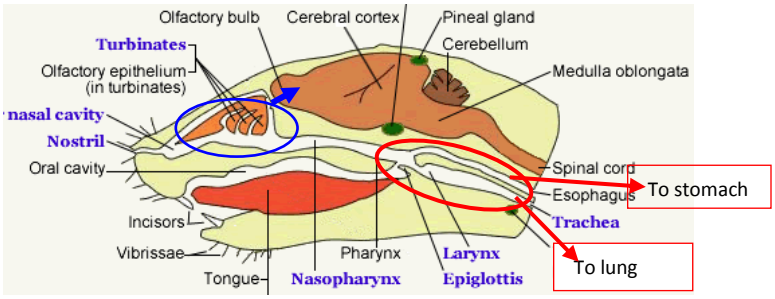
4. Compare impacts on brain GM1 levels and disease phenotype in *β-gal-/-* mice following nasal versus intravenous administration of β-gal:RTB.

   2-Year Milestone: *in vivo* data comparing administration routes (i.v. vs. nasal) in mediating CNS delivery of β-gal:RTB and reduction in brain pathologies.

Description of Experiments: Consistent with the proposed timeline, research in the first six months focused on Objective 1 resulting in the production and characterization of plant-made β-gal:RTB product with progress described in the previous report. This report focuses on Objective 2 which involves an initial administration of the β-gal:RTB product into the nostrils of *β-gal-/-* mice. β-gal:RTB was purified from leaf material and purity, endotoxin levels, β-gal enzyme activity, lectin binding, and concentration was determined for the product. We also demonstrated that the plant-made product purified as a dimer (~240 kDa) based on size exclusion chromatography and gel electrophoresis under non-reducing conditions. This product was used for nasal instillation into *β-gal-/-* mice.

Primary Results:

Nasal instillation procedures. The proposed initial *in vivo* assessment experiments involved nasal administration of the β-gal:RTB fusion protein and quantification of β-gal enzyme activity in various tissues at selected times after test-solution instillation. Nasal administration in mice is not trivial – the nostril is small and the anatomy of the mouse lends to rapid drainage to the lung or stomach which impacts the concentration that is accessible to transnasal transfer (see diagram). Prior to nasal instillation of β-gal:RTB test material β-gal:RTB mice, we tested administration procedures using a small-molecule dye administered to wildtype mice. Although mechanisms of small-molecule uptake into the brain are different than those mediating delivery of large proteins, the dye provides a readout of test-solution retention within the nasal cavity. The movement of dye into brain, lung and stomach was assessed at 1 hr. This system was used to optimize mouse position, volume per administration, timing between administrations, and logistics of managing nasal instillation for multiple mice. Conditions were identified that reduced dye distribution to the stomach and lungs and showed dye distribution to the brain.



Results of first transnasal experiment. As an initial assessment of transnasal delivery, *β-gal-/-* mice (6-9 weeks of age) were treated with β-gal:RTB instilled in a drop-wise manner alternating to each nostril with a lapse of several minutes between administrations. One hour after the final administration, mice were euthanized and tissues (lung, stomach, and brain subregions including olfactory bulb, mid brain, brain stem and cerebellum) were processed for enzyme activity assays. Levels of β-gal enzyme activity in extracted tissues of treated and non-treated β-gal-/- mice were compared. Increased β-gal activity levels were consistently observed in the cerebellum of treated mice (n=3) and in the brain stem and midbrain of 2 out of 3 treated mice. Interestingly, elevated β-gal activity was not observed in the olfactory bulb of treated animals, which is the region of the brain that would be initially accessed by protein transport involving the direct nose-to-brain mechanisms associated with the olfactory neural network. Detection of β-gal activity in the more distal regions (cerebellum, brain stem) may suggest transport via either the trigeminal nerve system or circulatory routes. Year 2 experiments will be designed to address these questions and to assess impacts of multiple nasal administrations β-gal:RTB on reduction of the GM1 disease substrate in *β-gal-/-* mice.

Summary: Aim 1 research successfully yielded the purified plant-made β-gal:RTB fusion product to initiate transnasal trials in mice (six-month report). Aim 2 research has now provided initial promising results suggesting that β-gal:RTB accesses brain tissues following nasal administration and supports year 2 studies to further delineate routes of delivery and to assess GM1 disease correction following multiple transnasal administrations in the *β-gal-/-* mice.

Conclusions, Future Work, and Publications:

In the first year of this project, we have met key Aim 1 and 2 milestones and are preparing for the multiple-administration intranasal mouse trials consistent with the goals and timeline of the proposal. In related work funded by an NIH SBIR grant, we have shown that our β-gal:RTB product is well tolerated in mice and appears effective in accessing cells and clearing GM1 substrate. These results provide a strong foundation to move forward with further intranasal trials as proposed in the NTSAD grant. As this is very early stage research, the work has not resulted in publication. However, a manuscript describing plant-based production and

characterization of the β-gal:RTB product and its ability to “correct” GM1 gangliosidosis in vitro using GM1 gangliosidosis patient fibroblasts has been submitted for publication.