PHARMACOLOGY STUDY OF AAV VECTORS FOR GENOME EDITING IN GSD 1a INFANTILE MICE

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Background: Glycogen storage disease type Ia (GSD 1a) presents as a liver metabolic disorder that is caused by the deficiency of glucose-6-phosphatase related to pathogenic variants in the *G6PC* gene. Similarly, to other inherited disorders of metabolism, gene replacement therapy is under development and limited by the rapid loss of non-integrating viral vectors from the liver early in life, such as adeno-associated virus (AAV) vectors. We have developed AAV vectors for genome editing to stably integrate a therapeutic *G6PC* transgene, mediated by CRISPR/Cas9 cleavage of the *G6PC* locus and homology-directed recombination (HDR). A Donor vector containing the *G6PC* transgene flanked by homology to the *G6PC* locus can be integrated in the presence of a CRISPR vector that contains *Streptococcus pyogenes* Cas9 and a small guide RNA targeting *G6PC*.

Methods: These two vectors were administered to 10 day old *G6pc* -/- mice with GSD Ia, and efficacy was evaluated in comparison with *G6pc* -/- mice that received only the Donor vector that would not integrate in absence of the CRISPR. The vectors were administered at low (Donor, 2E+12 vector genomes/kg; CRISPR 4E +11 vector genomes/kg), or medium dosages (each vector 4-fold higher). Benefits of genome editing were evaluated at 6 weeks of age, by comparison of the Donor + CRIPSR group to the Donor only group at each dose.

Results: Improved glucose homeostasis was demonstrated by the following statistically significant differences. Mice receiving both Donor + CRISPR vectors had increased blood glucose concentrations during fasting (low dose; $p<0.0001$) and decreased liver glycogen compared with mice receiving Donor vector only (medium dose; $p<0.001$). In the glucose tolerance test, Donor vector administration improved blood glucose at baseline following 4 hours fasting (low dose; $p<0.0001$) and at 120 minutes following glucose administration (low dose; $p<0.01$). Significantly more copies of the *G6PC* transgene were detected in the liver for the CRISPR + Donor treated group than for the Donor treated mice (medium dose; $p<0.05$). Additionally, the liver glycogen concentrations of the medium dose CRISPR + Donor treated mice was significantly lower than untreated *G6pc* -/- controls ($p<0.001$). After 4 weeks of treatment with both AAV vectors, the medium dose mice had liver glycogen concentrations in the range of wildtype mice. This study has been extended to include evaluation of the same groups at 14 weeks of age and evaluation of transgene integration.

Conclusions: We have demonstrated delivery of a CRISPR/Cas9 vector increases efficacy of our *G6PC* transgene over the transgene vector alone. Infant GSD 1a mice receiving the CRISPR to stimulate permanent integration of the *G6PC* transgene had higher blood glucose, increased copies of the transgene, and lower glycogen in liver. The CRISPR treated mice also had hepatic glycogen content similar to wildtype mice. None of these benefits were attributable to CRISPR/Cas9 expression, which has no known effects on glucose metabolism. This data suggests combination therapy with CRISPR/Cas9 helps prevent the loss of therapeutic AAV transgenes in young mice, and justifies the further, long-term evaluation of genome editing in GSD Ia.