Evaluation of Systemic Application of Adeno-associated Virus in Neonatal Rodents for the Development of Gene Therapy in Neonatal Hydrocephalus

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Introduction: Neonatal hydrocephalus leads to increased intracranial pressure from abnormal accumulation of cerebrospinal fluid in the ventricles of the infant brain that may arise from both genetic and acquired causes. Surgical treatment remains the standard of care as currently there is no cure for this condition. Furthermore, patients are at risk for long-term motor, cognitive, and behavioral deficits. Adeno-associated viruses (AAVs) targeting the central nervous system (CNS), such as AAV-F, make gene therapy a promising, non-surgical cure for hydrocephalus. The aim of this study was to determine the efficiency and timing of AAV-F delivery in neonatal mice and rats. This must be elucidated before pre-clinical gene therapy studies for hydrocephalus can be undertaken.

Hypothesis: Delivery of AAV-F expressing enhanced green fluorescent protein (AAV-F-EGFP) in mice and rats at early postnatal age will lead to robust expression of EGFP throughout the CNS, allowing for optimization of the therapeutic window and delivery route for gene therapy in neonatal mouse and rat models of hydrocephalus.

Methods: AAV-F and AAV-PHP.B (positive control) vectors were purified, packaged, and titrated. Viral vectors (doses of 2.40×10^{11} viral genomes (vg) or 3.23×10^{11} vg for neonatal mice or rats, respectively) were injected into the facial vein of postnatal day (P) 2 mice (C57BL/6 strain) and rats (Sprague Dawley strain) for systemic delivery. Animals were sacrificed one-week or three-weeks post-injection and processed for analysis of EGFP expression.

Results: Mice and rats injected with AAV-F-EGFP exhibit diffuse expression of EGFP in the brain by P9 relative to non-injected controls. Importantly, neurons and astrocytes in brain structures implicated in hydrocephalus pathophysiology, including the cortex and choroid plexus, show the most intense expression of the EGFP gene therapy product.

Conclusions: Successful CNS transduction of neonatal mouse and rat brains with AAV-F-EGFP supports AAV-F mediated-expression of transgenic proteins in brain structures that are dysfunctional in rodent models of hydrocephalus with genetic etiology. This study provides a new tool for the development of robust gene therapy as a non-surgical treatment for restoring healthy brain development in pre-clinical rodent models of hydrocephalus.

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