Intra-Arterial Viral Mediated Gene Therapy after Osmotic Blood Brain Barrier Disruption in Mice

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Introduction

One factor limiting the application of gene therapies to treat diseases of the central nervous system (CNS) is the lack of effective delivery techniques. Batten disease is an inherited neurodegenerative disease caused by a mutation in the CLN2 gene resulting in decreased levels of TTP-1 enzyme. This results in lipofuscin accumulation in the brain causing progressive loss of cognitive, motor and sensory function, ultimately leading to death after the first decade of life. Here we describe a novel method for intra-arterial delivery of AAVrh.10 CLN2 following osmotic blood brain barrier (BBB) disruption in mice.

Methods

The left internal carotid arteries of naïve mice were catheterized using a 169 µm diameter microcatheter. After intra-arterial mannitol injection, one of four AAVrh.10 CLN2 doses (3.175 x 10^10 gc, 6.35 x 10^10 gc, 1.27 x 10^11 gc, 2.54 x 10^11 gc) was administered (n=3 for each group). An intravenous mannitol with AAVrh.10 CLN2 (2.54 x 10^11 gc) group (n=3) was also done for comparison. Control groups included intra-arterial mannitol plus saline (n=3) or saline plus AAVrh.10 CLN2 (n=3). Animals were sacrificed at five weeks to assess the extent of TPP-1 production and compare IV vs. IA delivery (figures 1,2 and 4).

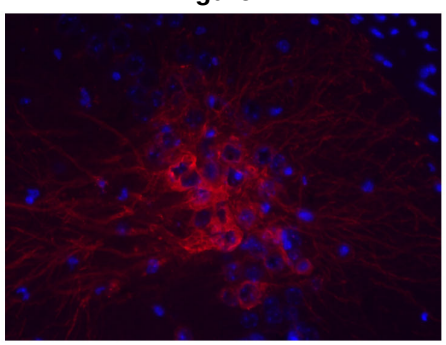
Results

All animals treated with IA mannitol and AAVrh.10 CLN2 showed TPP-1 production in left cerebral hemisphere by immunohistochemistry. Fluorescence activity assays for TPP-1 showed significantly higher levels of activity in the brain ($p<0.05$) and less systemic activity compared to IV treated animals (figure 4). Control animals receiving mannitol alone or AAVrh.10 CLN2 alone did not reveal any significant TPP-1 production.

Conclusions

In this study we demonstrate that co-administration of intra-arterial mannitol and AAVrh.10 CLN2 resulted in diffuse hemispheric TPP-1 production. Furthermore, TPP-1 was shown in both neurons and glial cells and was sustained at greater than 4 weeks after delivery. Intra-arterial gene therapy after osmotic disruption of the BBB may be a viable delivery method for viral-mediated gene therapy to the CNS.

Figure 1



40x fluorescent micrograph showing TTP-1 protein expression (red) in the hippocampus after intra-arterial delivery of AAVrh.10 CLN2 virus particles.

Figure 2A

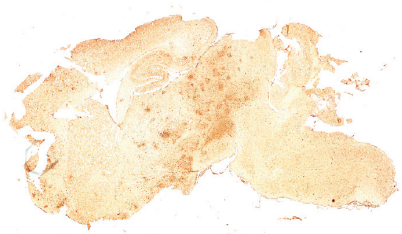
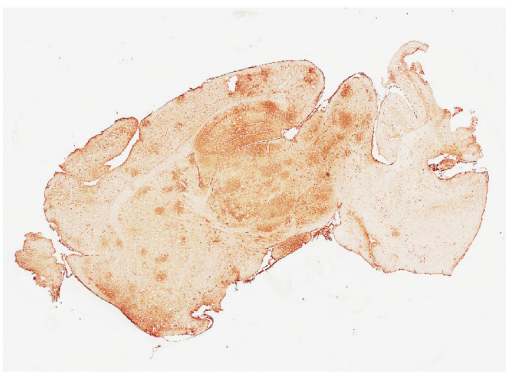
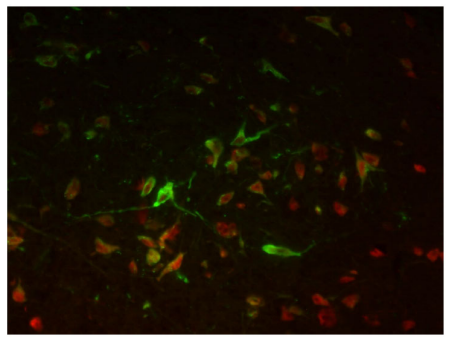


Figure 2B



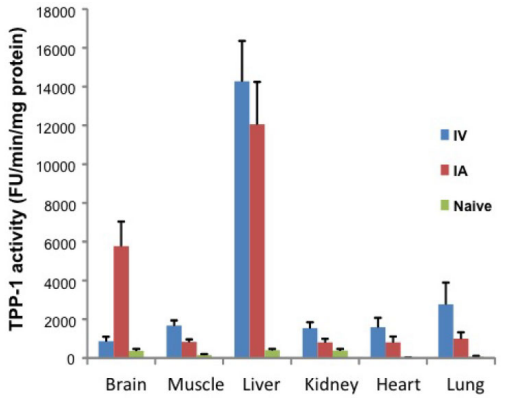
Immunohistochemistry showing TPP-1 (dark brown) production in (A) low- dose AAVrh.10 CLN2 (3.175 x 10^10) and (B) high-dose AAVrh.10 CLN2 (2.54 x 10^11) given intra-arterially following mannitol administration.

Figure 3



Fluorescent micrograph showing co-localization of TTP-1 with Fox-3 (neuronal marker) after intra-arterial delivery of AAVrh.10 CLN2 virus particles.

Figure 4



Brain and systemic activity of TPP-1 after IA and IV delivery of AAVrh.10 CLN2 following mannitol administration

Learning Objectives

By the conclusion of this session, participants should be able to discuss the delivery of AAVrh.10 CLN2 gene therapy via intra-arterial injection after osmotic blood brain disruption in mice.