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Commentary

Breaching the blood-brain barrier: AAV triggers dose-dependent toxicity in the brain

Daniel Stone,[1] Martine Aubert,[1] and Keith R. Jerome[1,2]

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Since *in vivo* transduction of neurons and glia by adeno-associated virus (AAV) vectors was first demonstrated in rat brain,[1] it has been shown that AAV can efficiently transduce neurons, microglia, astrocytes, and oligodendrocytes across multiple species, and AAV vectors have become a widely used tool in basic and therapeutic research targeting the nervous system.[2] However, recent observations have shown that the administration of AAV vectors at high doses can cause local or widespread neurotoxicity in the central (CNS) and peripheral (PNS) nervous systems, and the long-term consequences of this for human therapies targeting nervous tissue remain to be determined. In this issue of *Molecular Therapy Methods and Clinical Development*, Guo and colleagues investigate the mechanisms by which AAV mediates neurotoxicity following intraparenchymal injection into mouse brain.[3] Their observation that high doses of AAV can cause localized disruption of the blood-brain barrier (BBB), which allows an influx of blood and serum factors into brain parenchyma (Figure 1), provides important mechanistic insights into our understanding of how neurotoxicity at the site of injection is mediated by infiltrating lymphocytes.

Although endemic AAV infections have not been reported in nervous tissue, vectorized AAV shows broad neurotropism across many species. Generally, AAV is considered to be safe, and hundreds of studies have used AAV vectors to target the nervous system with few reporting neurotoxicity. Nevertheless, reports of neurotoxicity following delivery of high AAV doses have raised concerns about the long-term impact AAV therapies may have on neurological functions.[4] The mechanisms by which AAV triggers toxicity and/or cell death in nervous tissues are poorly understood, although immune-mediated mechanisms and apoptosis following activation of the unfolded protein response have been implicated in the death of AAV transduced neurons within the dorsal root ganglion (DRG).[5] In the brain, little is known about vector-mediated neurotoxicity, even though it has been observed in brains of mice, rats, and non-human primates (NHPs) (Table 1) and, more recently, in a clinical trial for late infantile Battens disease.[6] Importantly, vector-mediated neurotoxicity across different species has been observed when similar AAV doses are administered relative to the recipient brain size (6.43×10^8–2.5×10^{10} vector genomes [vg] per gram of brain tissue, Table 1).

In this manuscript, Guo et al. investigate the events that trigger localized neurotoxicity in mice at the site of injection after administration of two AAV doses (1×10^8 and 1×10^{10} vg/brain, equivalent to 2.33×10^8 and 2.33×10^{10} vg/gram of brain). They first show a dose threshold effect for activation of microglia and astrocytes at the higher dose around the injection site, and for activation of genes associated with anti-viral responses, recruitment of immune cells, BBB integrity, and inflammatory responses, via bulk RNAseq. They then show that localized disruption of vascular endothelial tight junctions, astrocytic perivascular endfeet, and pericyte-endothelial cell interactions occur at the higher dose between 10 and 30 days post injection (dpi), which permeabilizes the BBB and enables blood cells or serum proteins such as platelets and IgG to enter the parenchyma. BBB permeability at the site of AAV injection was also demonstrated after fluorescent cadaverine and dextran tracers were administered intravenously.

Next, the authors investigated whether BBB disruption facilitates immune cell infiltration and acute-neuronal cytotoxicity at the site of high-dose AAV administration. CD4+, CD8+, and CD19+ lymphocytes infiltrated the injection site between 6 and 10 dpi, persisting until 30 dpi, and CD4+/GranzymeB+ T cells were found at the injection site at 20 and 30 dpi along with TUNEL+/NeuN+ neurons. After high-dose AAV administration, progressive loss of NeuN+ cells was observed at the injection site from 10 to 30 dpi. BBB disruption and immune cell influx was also seen when high AAV doses were delivered to the striatum and hippocampus, following AAV5 or AAV9 delivery, and when different GFAP promoters were utilized, but not when empty capsids were administered. Interestingly, the loss of NeuN+ cells was independent of transgene expression in neurons since expression was restricted to astrocytes/glia by the GFAP promoter. In fact, the authors showed that expression of the GFP transgene was not required for BBB disruption, immune cell infiltration, or loss of NeuN+ cells, which were all seen at the injection site in both wild-type and GFAP-Cre transgenic mice administered a Cre-lox AAV vector system that requires Cre expression to turn on transgene expression.

Finally, the authors investigated the effect of immune cell depletion on responses to high AAV doses in brain parenchyma. Treatment with anti-CD4 or anti-CD8 depleting antibodies inhibited both the influx of CD4+ and CD8+ T cells and the depletion of NeuN+ cells at the site of AAV injection. Similarly, treatment with an inhibitory anti-GranzymeB antibody partially attenuated loss of NeuN+ cells at the site of

[1]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Center, Seattle, WA, USA; [2]Department of Laboratory Medicine and Pathology, University of Washington, Seattle, WA, USA

Correspondence: Daniel Stone PhD, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Center, 1100 Fairview Ave N, Seattle WA 98109, USA.
E-mail: dstone2@fredhutch.org

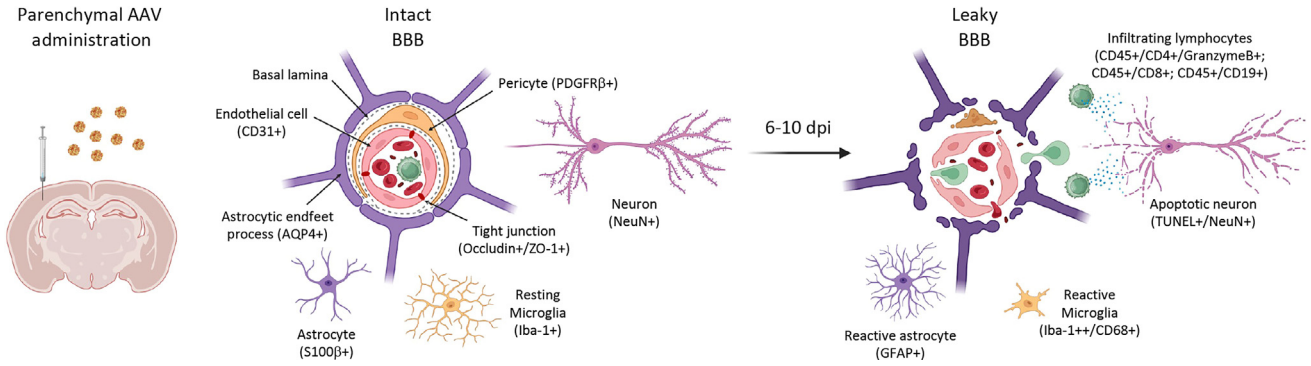

Figure 1. AAV-mediated blood-brain barrier disruption (BBB)

Following parenchymal AAV administration, astrocytes and microglia become activated at the site of injection, and the BBB is disrupted locally. BBB disruption enables local influx of inflammatory cells including CD45+/CD4+/GranzymeB+, and CD45+/CD8+ lymphocytes that mediate neuronal cell loss. Figure was created using biorender.com.

injection. These data suggest that infiltrating lymphocytes play a significant role in vector-mediated brain neurotoxicity and provide a potential approach to prevent unwanted neurotoxicity in future studies targeting brain parenchyma.

While these observations offer us a basic understanding of the biological processes that lead to neuronal death in the brain, much remains unknown about this phenomenon or its clinical significance. It is unclear how AAV triggers disruption of the BBB—is BBB disruption a direct or indirect consequence of AAV administration? And, why is BBB disruption not seen until after 6 dpi? The consequences of vector-mediated neurotoxicity on long-term neurological functions are also uncertain, both in the CNS and PNS. Abnormal neurological behaviors or deficits have been seen in some NHP with loss of neurons in the DRG or brain after systemic or parenchymal AAV administration respectively.[7–9] However, signs of neurotoxicity in the PNS or CNS were not associated with abnormal neurological behaviors or deficits in most animals. It is worth noting that abnormal DRG pathology in NHPs is largely not detectable by 6 months post AAV administration,[8] whereas the long-term persistence of vector-associated neurotoxicity in NHP brain has not been studied in depth. The relevance of these observations to the clinical setting should also be determined, since lower AAV doses that are not neurotoxic may show therapeutic efficacy. Although limited, there is evidence that parenchymal neurotoxicity occurs in the human brain. In the trial for late infantile Battens disease, disease progression was slowed, but some trial participants had AAV injection site abnormalities detectable by MRI that persisted for up to 18 months, and adverse events including seizures, dystonia, and abnormal movements were seen in some participants.[6] Interestingly, trial participants received a slightly lower dose per brain weight than other species with reported vector-mediated parenchymal neurotoxicity (Table 1), demonstrating that the dose threshold for AAV-mediated BBB dysregulation may vary by species. This is highly relevant since calculations of the maximum permissible "safe" dose based on studies conducted in animals may not be accurate. Ultimately, the clinical significance of localized BBB disruption at high AAV doses should be studied further in animals and humans across a broader range of doses, and it will be important to longitudinally monitor neurological behaviors of patients receiving intra-cranial AAV administrations as therapy.

Table 1. Approximate AAV dose relative to brain weight for studies reporting localized neurotoxicity after intra-parenchymal delivery of AAV vectors

Study	Species – strain	AAV dose administered (vg/brain)	Assumed brain weight (g)	AAV dose (vg/g of brain)
Guo et al.[3]	mice – C57BL/6	1×10^{10}	0.43	2.33×10^{10}
Johnston et al.[10]	mice – C57BL/6	3×10^{9}	0.43	7×10^{9}
Klein et al.[11]	rats – Sprague Dawley	5×10^{10}	2	2.5×10^{10}
Rosenberg et al.[12]	African green monkey	1.5×10^{12}	64.3/61.1, M/F	$2.33–2.45 \times 10^{9}$
Samaranch et al.[13]	*Macaca fascicularis*	6×10^{11}	68.4/60.4, M/F	$8.8–9.9 \times 10^{9}$
Golebiowski et al.[7]	*Macaca fascicularis*	3.2×10^{12}	68.4/60.4, M/F	$4.7–5.3 \times 10^{10}$
Keiser et al.[9]	*Macaca mulatta*	$>6 \times 10^{11}$	86.1/96.1, M/F	$>6.24–6.97 \times 10^{9}$
Sondhi et al.[6]	human	9×10^{11}	1,000[a]	6.43×10^{8}

The lowest dose at which parenchymal neurotoxicity was seen is reported.
[a]Approximate brain weight of a 1- to 5-year-old child.

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DECLARATION OF INTERESTS

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