Title

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Journal

Stroke, 44(1)

ISSN

0039-2499

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Publication Date

2013

DOI

10.1161/strokeaha.112.662965

Peer reviewed

Published in final edited form as:
Stroke. 2013 January ; 44(1): 252–254. doi:10.1161/STROKEAHA.112.662965.

Intravenous delivery of AAV9 vector mediates effective gene expression in ischemic stroke lesion and brain angiogenic foci

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Abstract

Background and Purpose—Adeno-associated viral vector (AAV) is a powerful tool for delivering genes to treat brain diseases. Intravenous delivery of a self-complementary, but not single-stranded, AAV9 vector (ssAAV9) mediates robust gene expression in the adult brain. We tested if ssAAV9 effectively mediates gene expression in the ischemic stroke lesion and angiogenic foci.

Methods—Focal ischemic stroke was induced by permanent occlusion of the left middle cerebral artery (MCAO), and focal angiogenesis, by injecting an AAV vector expressing vascular endothelial growth factor (AAV-VEGF) into the basal ganglia. ssAAV vectors that have CMV promoter driving (AAV-CMVLacZ) or hypoxia response elements controlling (AAV-H9LacZ) LacZ expression were packaged in AAV9 or AAV1 capsid, and injected into mice through the jugular vein one hour after MCAO or four weeks after the induction of angiogenesis. LacZ gene expression was analyzed in the brain and other organs five days post LacZ vector-injection.

Results—LacZ expression was detected in the peri-infarct region of AAV9-CMVLacZ and AAV9-H9LacZ-injected MCAO mice, and the brain angiogenic foci of AAV9-CMVLacZ-injected mice. Minimum LacZ expression was detected in the brain of AAV1-CMVLacZ-injected mice. Robust LacZ expression was found in the liver and heart of AAV-CMVLacZ-injected mice, but not AAV9-H9LacZ-injected mice.

Conclusion—ssAAV9 vector could be a useful tool to deliver therapeutic genes to the ischemic stroke lesion or brain angiogenic foci.

Keywords

peri-infarct region; AAV serotype 9; mouse; brain; angiogenesis; intravenous delivery

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Disclosures
None.

Introduction

AAV is an ideal vector for delivering genes into the brain because it effectively infects neurons and astrocytes. It has been used to deliver genes to various brain disease models.[1, 2] In most studies, the AAV vectors were delivered to the brain via stereotactic injection. Direct injection, however, is an invasive procedure that can cause additional damage to a critically ill patient.

Recombinant AAV packaged in a serotype 9 capsid effectively passes through the blood-brain barrier (BBB).[3, 4] However, only self-complementary AAV9 vector (scAAV9), not single stranded AAV9 vector (ssAAV9), robustly mediates transgene expression in the adult brain after intravenous (IV)-injection.[5] Many therapeutic genes are too big to be packaged as scAAV. We demonstrate in this study that IV-injected ssAAV9 (AAV-CMVLacZ) can effectively deliver genes into the adult brain in the ischemic peri-infarct region and angiogenic foci. AAV9 vector with hypoxia response elements (HREs) (AAV-H9LacZ) restricts gene expression specifically in the peri-infarct region of the brain with focal ischemic injury.

Materials and Methods

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco, and conformed to NIH Guidelines for use of animals in research. CD1 male mice at age 8 to 10 weeks (Charles River, Wilmington, MA) were used.

Focal Ischemic Stroke Model and Brain Angiogenic Model

Focal ischemic stroke was created by permanent occlusion of the left distal middle cerebral artery (MCAO).[2] Brain focal angiogenesis was induced by stereotactic injection of AAV-VEGF, 2×10^9 genome copies (gcs), into the basal ganglia.[6]

IV-injection of AAV vectors

Into the jugular vein, AAV-H9LacZ and AAV-CMVLacZ[7] (2×10^{11}, 8×10^{11}, 1×10^{12} gcs) in 200 μL PBS were injected one hour after MCAO (N=6) and four weeks after induction of angiogenesis (N=6).

Additional methods are described in the online-only Data Supplement (available at http://stroke.ahajournals.org.)

Results

Stroke Model

AAV9-CMVLacZ or AAV1-CMVLacZ was injected into the jugular vein one hour after MCAO (Figure 1A). Brain samples were collected five days later. Infarct region was visualized on Nissl-stained and NeuN antibody-stained sections (Figure 1B, Supplemental Figure S3). LacZ expression in the brain was predominantly in the peri-infarct region of AAV9-CMVLacZ-injected mice and was very weak in other brain regions (Supplemental figure S7). No LacZ expression was detected in the brain of AAV1-CMVLacZ-injected mice, including the peri-infarct region (Figure 1C). LacZ expression was detected in the heart and liver of all mice injected with AAV1- or AAV9-CMVLacZ (Supplemental Figure S1).

We then tested if HREs could prevent gene expression in other organs. AAV9-H9LacZ that has 9 copies of HREs controlling LacZ expression[7] was injected into the jugular vein one

hour after MCAO. LacZ expression was detected only in the peri-infarct region five days later (Figure 1B & C). No significant gene expression was detected in other brain regions and other organs (Supplemental Figure S1 & S7).

The infarct size and the number of $CD68^+$ cells at the peri-infarct region were comparable among non-vector injected and vector-injected mice (Supplemental Figures S4 & S5), suggesting that IV-delivered AAV vector did not increase local inflammation and neuronal injury.

Angiogenic Model

Brain focal angiogenesis was induced by stereotactic injection of AAV1-VEGF into the basal ganglia. AAV9-CMVLacZ or AAV1-CMVLacZ was injected into the jugular vein 28 days later. LacZ positive spots were found predominantly in the angiogenic foci five days later in the AAV9-CMVLacZ group, but not in the AAV1-CMVLacZ group (Figures 2B & C). LacZ expression was also detected in the heart and liver of all mice (Supplemental Figure S2).

Discussion

We demonstrated that (1) IV-injection of ssAAV9 mediates significant transgene expression in the peri-infarct region of focal ischemic injury and brain angiogenic foci, and (2) HRE restricted transgene expression in the peri-infarct region. Therefore, ssAAV9 combined with regulated elements can mediate targeted therapeutic gene expression in the brain lesion through non-invasive IV-injection.

Active transport mechanism has been suggested in facilitating AAV9 crossing BBB.[8] In our study, however, higher LacZ expression was detected in the peri-infarct region and angiogenic foci than in other brain regions, suggesting that increased BBB permeability plays an important role. The BBB permeability is increased within 10 minutes after permanent MCAO and the increase lasts at least 24 hours (Supplemental Figure S6).[9, 10] However, we do not know if the expression pattern persists when the vectors are injected at a later stage of MCAO.

Although IV injection of ssAAV9 (5×10^{11} gcs) infects some cells in the normal brain, the efficiency is much lower than that of scAAV9.[8] We showed that after IV injection, gene expression in the brain is predominantly at the peri-infarct area and angiogenic foci. A few LacZ positive cells in the contralateral brain of mice received 8×10^{11} and 1×10^{12} gcs of AAV9-CMVLacZ (Supplemental Figure S7), which is similar to what Gary et al found in their study with 5×10^{11} gcs ssAAV9-GFP.[8]

In summary, we have demonstrated in this study that IV injection of ssAAV9 can deliver therapeutic genes into the brain regions of adult mice where the BBB permeability is increased. More importantly, to reduce systemic side effects, the therapeutic gene expression can be further restricted to the brain lesion by incorporating regulatory elements. ssAAV9, in combination with regulator elements, can be used to design safe and effective gene-based therapies for the treatment of ischemic stroke and brain vascular diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Voltaire Gungab for assistance with manuscript preparation, Osman Nacar and Uday Sarvepalli for assistance in tissue sections, and the other members of the UCSF BAVM Study Project (http://avm.ucsf.edu) for their support.

Sources of Funding

This study was supported in part by grants from the National Institutes of Health (R01 NS027713 to W.L.Y., P01 NS044155 to W.L.Y. and H.S., and R21 NS070153 to H.S.), and from the American Heart Association (AHA 10GRNT3130004 to H.S.).

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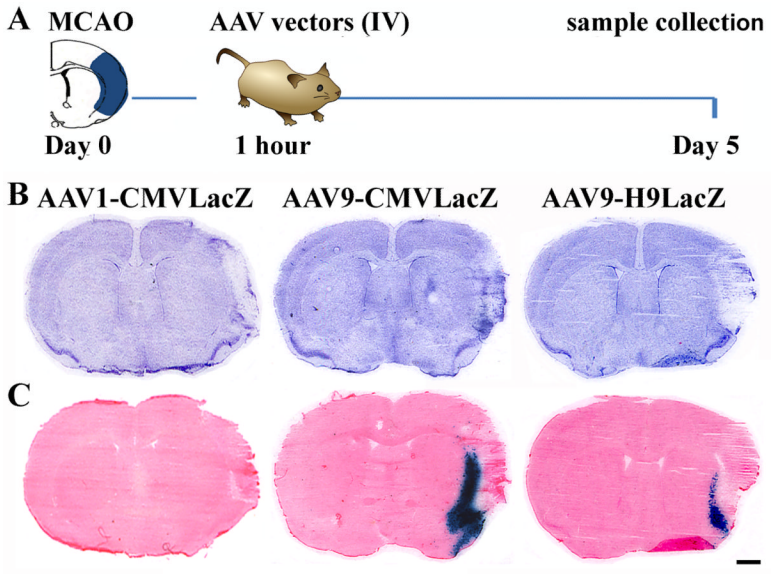

Figure 1. LacZ expression in the peri-infarct region

(A) Experimental design. (B) Representative Nissl-stained sections showing infarct area. (C) Representative image of X-gal-stained sections showing LacZ expression in the peri-infarct region. No LacZ expression was detected in the brain of AAV1-CMVLacZ-injected mice. Scale bar: 1mm.

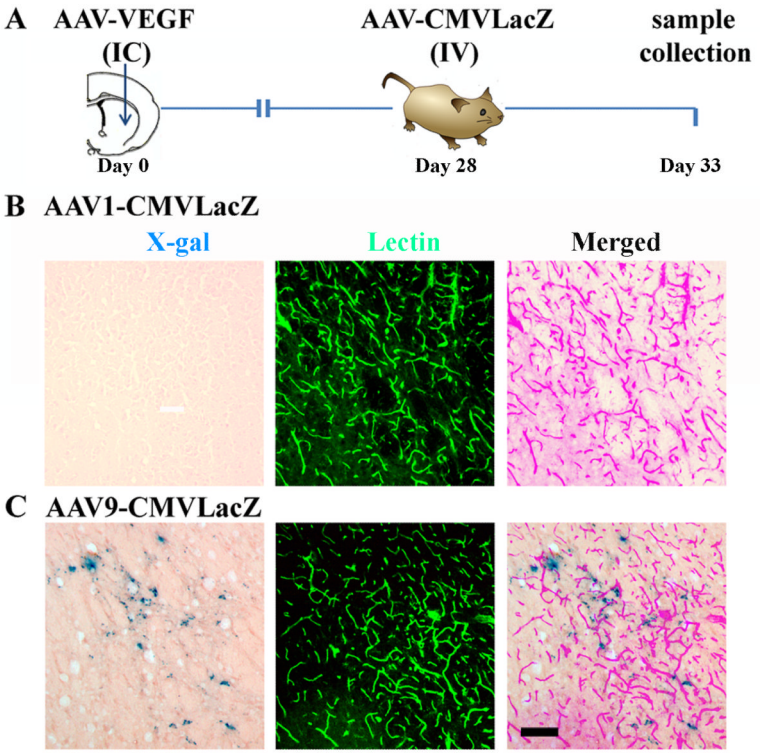

Figure 2. LacZ expression in angiogenesis foci

(A) Experimental design. (B & C) Representative images of a lectinX-gal (left) and lectin (middle) co-stained brain section collected from AAV1CMVLacZ (B) AAV9-CMVLacZ (C) vector-injected mice with brain angiogenesis. To visualize both vessels and LacZ expression, lectin positive vessels were converted to pink in the merged picture (right). Scale bar: 100 μm.