Intravenous Delivery of Adeno-Associated Viral Vector Serotype 9 Mediates Effective Gene Expression in Ischemic Stroke Lesion and Brain Angiogenic Foci

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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 (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 was induced by injecting an AAV expressing vascular endothelial growth factor (AAV-VEGF) into the basal ganglia. ssAAV vectors that have cytomegalovirus (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 1 hour after MCAO or 4 weeks after the induction of angiogenesis. LacZ gene expression was analyzed in the brain and other organs 5 days after 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 in AAV9-H9LacZ–injected mice.

Conclusions—ssAAV9 could be a useful tool to deliver therapeutic genes to the ischemic stroke lesion or brain angiogenic foci. (Stroke. 2013;44:00-00.)

Key Words: adeno-associated viral vector serotype 9 ▪ angiogenesis ▪ brain ▪ intravenous delivery ▪ mouse ▪ peri-infarct region

Adeno-associated viral vector (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 AAVs 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 (AAV9) capsid effectively passes through the blood–brain barrier (BBB).3,4 However, only self-complementary AAV9 (scAAV9), not single-stranded AAV9 (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 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 National Institutes of Health Guidelines for use of animals in research. CD1 male mice at age 8–10 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).6 Brain focal angiogenesis was induced by stereotactic injection of AAV expressing vascular endothelial growth factor (VEGF; AAV-VEGF), 2 × 10⁹ genome copies (gcs) into the basal ganglia.6
**Result**

*Stroke Model*

AAV9-CMVlacZ or AAV1-CMVlacZ was injected into the jugular vein 1 hour after MCAO (Figure 1A). Brain samples were collected 5 days later. Infarct region was visualized on Nissl-stained and NeuN antibody-stained sections (Figure 1B). Detailed Methods are provided in the online-only Data Supplement.
and Supplementary Figure III). 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 (Supplementary Figure VII). 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-CMVlacZ or AAV9-CMVlacZ (Supplementary Figure I).

We then tested if HREs could prevent gene expression in other organs. AAV9-H9lacZ that has 9 copies of HREs controlling LacZ expression was injected into the jugular vein 1 hour after MCAO. LacZ expression was detected only in the peri-infarct region 5 days later (Figure 1B and 1C). No significant gene expression was detected in other brain regions or other organs (Supplementary Figures I and VII).

The infarct size and the number of CD68+ cells at the peri-infarct region were comparable among nonvector-injected and vector-injected mice (Supplementary Figures IV and V), 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 5 days later in the AAV9-CMVlacZ group, but not in the AAV1-CMVlacZ group (Figure 2B and 2C). LacZ expression also was detected in the heart and liver of all mice (Supplementary Figure II).

### Discussion

We demonstrated that IV injection of ssAAV9 mediates significant transgene expression in the peri-infarct region of focal ischemic injury and brain angiogenic foci, and that 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 noninvasive IV injection.

Active transport mechanism has been suggested in facilitating AAV9 crossing the BBB. 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 (Supplementary Figure VI). 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 ssAAV9. 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 gcs and 1 × 10^12 gcs of AAV9-CMVlacZ (Supplementary Figure VII), which is similar to what Gary et al found in their study with 5 × 10^11 gcs ssAAV9-green fluorescent protein.

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.

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### Disclosures

None.

### References

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