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Purpose
The blood-brain barrier (BBB) is a significant barrier to viral gene therapy of brain tumors. We used imaging to investigate the effectiveness of BBB disruption to increase the delivery of therapeutic viral vector to the brain, using a radiolabeling technique to map and quantitate the distribution of genetically engineered virus.

Materials & Methods
Nude rats (nu/nu) (n = 12, 4/group) were implanted with bilateral Gl36 brain tumors in the frontal lobes. At 7 days after implantation, animals were injected with genetically engineered Herpes Simplex Virus (HSV) radiolabeled with 111Indium-oxine. Three routes of administration were used: a) intravenous (iv), b) intraarterial (IA) into the right carotid artery, and c) intraarterial into the right carotid with disruption of the BBB. Those animals undergoing BBB disruption were treated with a combination of intraarterial 20% Mannitol (2 mL/100 g) and Bradykinin (20 ug total dose divided into pre and postvirus injections by slow intraarterial injection). Nuclear imaging was performed on the animals during the injection and continuing for 1 hour, after which the animals were sacrificed, and the brains removed. The tumors, a border zone around each tumor, and the normal brain tissues were dissected out and counted using a gamma counter. The microscopic distribution of virus was studied using autoradiography in a single animal after BBB disruption.

Results
Imaging demonstrated no significant difference between the right and left hemispheres for those animals receiving intravenous virus. There was a consistent right-left (R/L) difference between those animals receiving IA virus by dynamic ROI analysis. Those animals undergoing BBB disruption showed a greater average R/L difference, but greatly increased variability between individual animals. Organ counts confirmed the right and left brain to have similar relative activities for animals receiving iv virus (99.3 &$\pm$177; 4.9% R/L), with greater differences for the IA group (108.9 ± 25.6% R/L) and BBB disruption group (172.2 ± 123.6% R/L), but with progressively greater variability. The total dose to the brain was 0.268 ± 0.027% of the injected dose (%ID) for the IV group, 0.30 ± 0.025% ID for the IA group and 0.384 ± 0.134% ID for those animals that underwent BBB disruption. The dissected brain parts counted separately revealed that most of the right-left differences were due to a relative increase of viral mass distribution to the normal brain parenchyma ipsilateral to the injection. This held for the IA group, and to a greater extent for the group undergoing BBB disruption. The variability in
delivery is also largely due to interindividual differences in delivery specifically to the normal brain. Autoradiography demonstrates a peculiar pattern of peripheral hot spots of increased delivery around the tumor, on a background of diffusely increased delivery to the right hemisphere.

**Conclusion**

1. The amount of virus delivered on average to the right vs left brain trends to increase progressively with more targeted delivery from IV, to IA to BBB disruption. 2. There is a large amount of interindividual variability in the efficacy of BBB disruption, ranging from highly effective disruption, to no effect at all. The reasons for this variability are not understood at present, but bradykinin has been reported previously to be ineffective as a BBB disruptor in the nude rat model, while mannitol was effective in 2. The combination of drug has not been tested previously to our knowledge. 3. BBB disruption has its greatest effect, not on the tumor, but on the surrounding brain. This may have therapeutic implications for treating gliomas, which typically recur at the resection margins, in areas of brain previously thought to be normal. 4. The microscopic distribution of virus is highly heterogenous, and to the tissue surrounding the tumor, rather than to the tumor itself. 5. Much further investigation will be required to confirm and explain these findings.

**References**