Magnetic resonance imaging of viral particle biodistribution in vivo

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Introduction: Gene transfer for therapeutic purposes is developing rapidly, and there is a clearly emerging need for a robust, non-invasive technique to detect viral vectors *in vivo*. Conventional imaging techniques for assessing viral biodistribution, such as immunohistology or polymerase chain reaction (PCR) are sensitive, but highly laborious. They also lack the non-invasive real-time option that would be especially important in clinical work. Considering the important role of magnetic resonance imaging (MRI) in current medical imaging, we wanted to explore the possibility of visualizing viral vector particle biodistribution in vivo by MRI. For this purpose, an avidin-displaying baculoviral vector (Baavi) was used [1]. We also sought proof that contrast agent labeling would not affect the transfection or homing capability of the avidin-coated baculoviral vector.

Methods: The avidin displaying baculoviruses (Baavi) harboring the lacZ gene were conjugated with biotinylated ultra-small (50 nm) superparamagnetic iron oxide particles (bUSPIO). The attachment of contrast agent particles to viruses was confirmed by atomic force microscopy (Vecco Instruments, CA, USA). bUSPIO+Baavi conjugates were then injected stereotactically into the right lateral ventricles of anesthetized female BDIX rat brain (n=5). Total amount of administrated iron was 0.5 µg, with $2.5*10^8$ infective viral particles per animal. One set of animals received bUSPIO only (n=4). MRI was performed at 4.7T, on a Varian UNITY/INOVA console (Varian Inc., CA, USA), using a $(35mm)^2$ FOV, using TE=10 ms and TR=0.32 s for GE images, and TE=75 ms, TR=2.0 s for adiabatic SE images. Animals were anesthetized with 1 % halothane in 74%:25% (N_2O:O_2) and fastened to a stereotactic holder. Animal core temperatures were maintained close to 37°C using a water-heated pad. Timepoints of 2 hours (0), 1, 3, 6, and 14 days after injection *in vivo* were studied. One subset of animals (n=4) was sacrificed five days after injection for histology.

Results: bUSPIO conjugation with Baavi was demonstrated by atomic force microscopy (Fig. 1). Baculoviruses exhibit cell-type specificity in the rat brain, with a tendency to home into choroids plexus cells. MRI contrast was detectable in the choroid plexuses of the ipsilateral ventricles (Fig. 2). No specific MRI signal changes were detected on the contralateral side (Fig. 2), nor when wild-type baculoviruses or plain biotinylated USPIO particles were injected into the lateral ventricles (data not shown). The contrast persisted in the ipsilateral ventricle for 11-14 days. Cryosectioned brains were stained for nuclear targeted β-galactosidase and DAB-enhanced Prussian Blue to detect iron (Fig. 3). LacZ gene expression was found to co-localize with the MRI contrast.

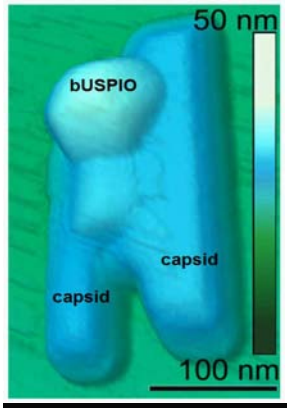
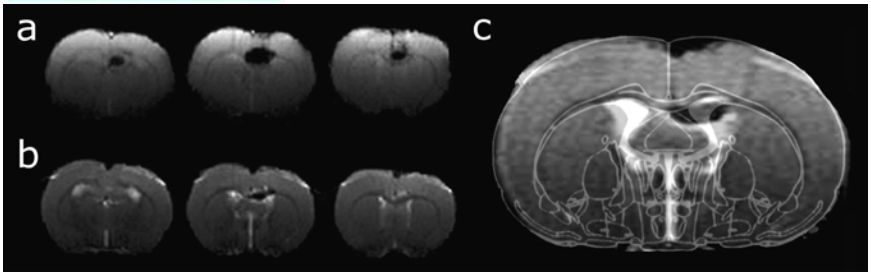


Figure 1 (to the left) *Presentation of bUSPIO-conjugated avidin-displaying baculovirus (Baavi). bUSPIO-attachment to viral particles was confirmed by atomic force microscopy. In-plane scale bar is shown at bottom, out-of-plane scale (height) is demonstrated by sliding color scale.*

Figure 2 (below) *MRI of intraventricular bUSPIO+Baavi delivery. Labeled viruses show as a dark region in the right ventricle. Sequential T2*-weighted GE (**a**) and adiabatic SE (**b**) MR images 2 hours after intraventricular bUSPIO-labeled Baavi injection. Although sensitivity is lower, anatomical features are better delineated in the SE image. Anatomical reference map (Paxinos et al.) is shown together with MRI of Baavi + bUSPIO from one animal (**c**).*



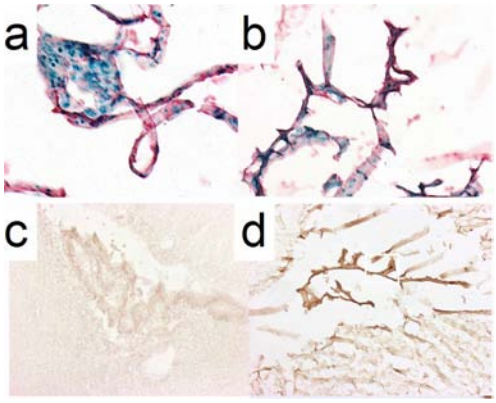


Figure 3 (above) *Choroid plexus histology. LacZ-marker gene expression in cells can be seen as a blue stain in cryosections five days after injection with wild type baculovirus (**a**) and bUSPIO+Baavi (**b**). There is now difference in transfection efficiency. DAB-enhanced Prussian-blue iron stainings demonstrate no detectable iron in the choroids plexus cells of rats injected with bUSPIO only (**c**), but positive ipsilateral staining in cells of animals injected with bUSPIO+Baavi (**d**).*

Conclusions: Our study provides proof-of-principle for robust and non-invasive viral vector imaging by MRI using an USPIO-labeled, avidin-displaying baculovirus *in vivo*. The utility of MRI is thus expanded also to the very initial steps of therapeutic gene delivery. Modifications of this labeling approach and different vectors are currently being investigated in our laboratory.

References: 1. Raty et al. (2004) Enhanced gene delivery by avidin-displaying baculovirus. *Mol.Ther.* 9: 282-291.