Case Study

The Role of Cryo-Fluorescence Tomography in Analyzing AAV7 & AAV9 Whole-Body Vector Biodistribution

Introduction

A recent study "Characterization and biodistribution of under-employed gene therapy vector AAV7" published by REGENXBIO, INC. in the Jounral of Virology explored the potential of AAV7 as a gene therapy vector and compared its biodistribution with AAV9 in mice following intravenous administration[1]. Cryo-Fluorescence Tomography (CFT) was used extensively in this research to analyze whole-body vector biodistribution and to guide immunohistochemistry (IHC) and PCR methods.

CFT was performed with EMIT Imaging's Xerra™ platform and was used to visualize the biodistribution of AAV7 and AAV9 carrying either an EGFP transgene or co-administration of AAV7.CAG.EGFP and AAV9.CAG.tdTomato, which highlights the ability to perform multi-fluorophore studies.

Key findings from CFT:

1. Results correspond to and expanded upon published AAV7 and AAV9 studies, for example weak biodistribution to the brain;

2. In the spinal trigeminal tract, AAV9 transduction was 1.5x higher than AAV7;

3. Skeletal muscle analysis further emphasizes increased expression by transduction with AAV9;

4. In the forelimb, AAV9-treated animals expressed EGFP 4.9x more than AAV7;

5. Heart transduction was higher in the AAV7-treated group (Fig. 1 A, C; Fig. 4A, B). These differences are best visualized in the dual-vector administration animals, where expression within the same animal is represented by AAV7 in green and AAV9 in red (Fig. 1B, C);

6. Unexpected biodistribution patterns in the facial structures, mandible, and sinus tissues, which had not been identified utilizing other biodistribution techniques. This is due to the ability to image in 3D and identify both on- and off-target tissues. In particular, the mandible showed the highest intensity of EGFP fluorescence of any other tissue quantified for both AAV7 and AAV9 (Fig. 1A, B).

Figure 1.

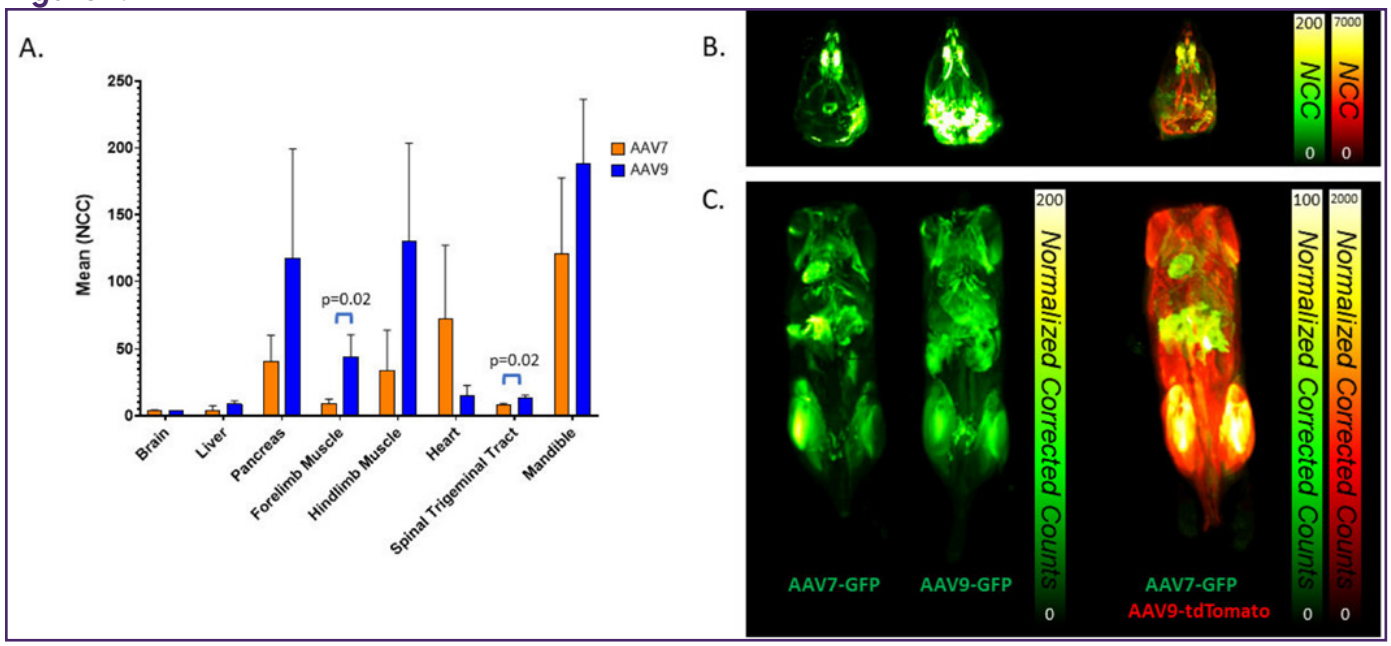

Fig. 1 (Fig. 5 from publication): CFT analysis of C57BL/6 mouse tissues 3 weeks after IV administration of 2 × 1014 GC/kg AAV7 and AAV9 carrying a CAG.EGFP transgene (n = 3). (A) Quantitative normalized corrected count (NCC) was determined by measurement within fixed volumes placed in organs. (B, C) Representative reconstructed maximum intensity projections of animals administered single vectors (left) or both AAV7.CAG.EGFP and AAV9.CAG. tdTomato(right).

Fig. 2 (Fig. S2A from publication) (video) - jvi.01163-23-s0002.mp4

Using the fiducial markers embedded in the block, planar fluorescent images were aligned and reconstructed into a 3D image, visualizing the AAV-mediated expression of the fluorescent biomarkers across the entire head. One representative animal per group is shown. The reconstructed samples are presented as maximum intensity projections (MIP). GFP is scaled at 0-200 NCC, tdTomato is scaled at 0-7000 NCC.

Fig. 3 (Fig. S2B from publication) (video) - jvi.01163-23-s0003.mp4

Using the fiducial markers embedded in the block, planar fluorescent images were aligned and reconstructed into a 3D image, visualizing the AAV-mediated expression of the fluorescent biomarkers across the entire body. One representative animal per group is shown. The reconstructed samples are presented as maximum intensity projections (MIP). GFP is scaled at 0-200 NCC, tdTomato is scaled at 0-7000 NCC.

Figure 4.

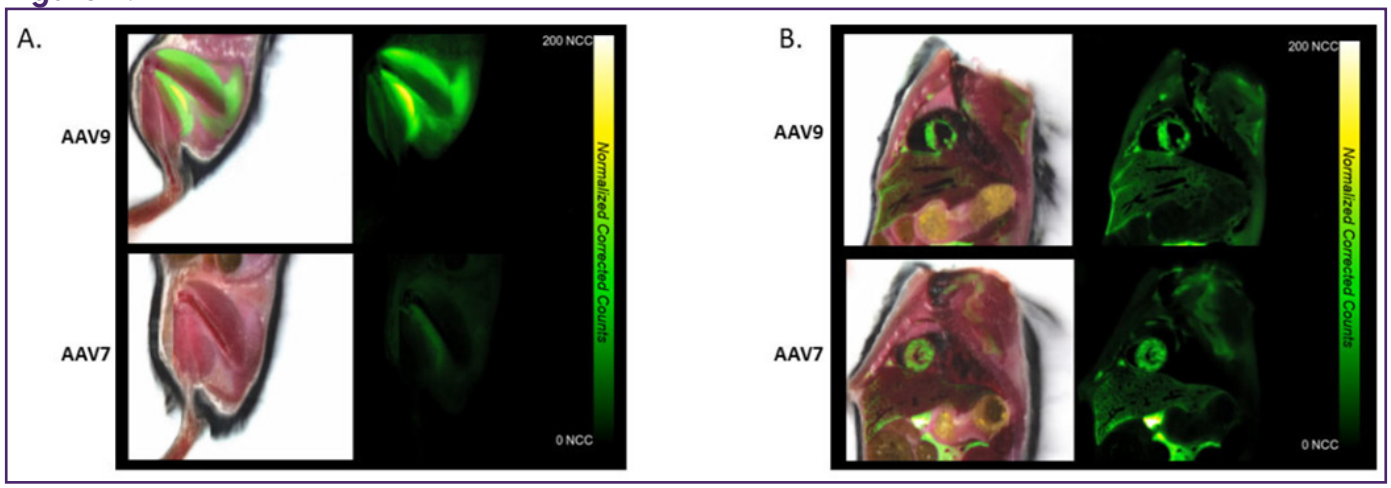

Fig. 4 (Fig. 6 from publication): Muscle transduction by AAV9 (top) and AAV7 (bottom). Representative CFT images of EGFP fluorescence (right) in hind limb muscle (A) and heart (B) overlaid on white light images (left).

The Role of CFT in the Study

CFT was instrumental in providing unbiased characterization of AAV7 and AAV9 in mice via whole-body detection and visualization of vector-mediated gene expression with high resolution and sensitivity. CFT significantly contributed to this study:

1. Unbiased Visualization of Vector Biodistribution: CFT showed localized vector biodistribution, enabling whole-body detection of protein expression in target and non-target organs. CFT overcomes sensitivity and resolution limitations implicit in *in vivo* imaging modalities.

2. Identification of Unexpected Tissue Tropism: CFT identified unexpected biodistribution patterns, such as the strong transgene expression throughout the head, including regions of the cranial sinuses, teeth, and mandible for both AAV7 and AAV9, which had not been previously evaluated.

3. Validated by Traditional Biodistribution Studies: CFT findings were supported by IHC and PCR methods; demonstrating the immense value that CFT provides in elucidating drug distribution and protein expression (PK/PD).

Future Opportunities

The use of CFT in this study sheds light on the unique biodistribution patterns of AAV7 and AAV9 and highlights the differentiated potential of AAV7 for tissue targeting. Specifically, CFT demonstrated the potential of AAV7 for strong cardiac tropism that differentiates it from AAV9. Such observations can be leveraged to seek modified and optimized AAV7 vectors that refine PK/PD relative to the therapeutic goal. Importantly, CFT can be the primary screening tool that guides this critical research. The ability of CFT to visualize target and non-target tissues and provide guidance to secondary biochemical methods highlights its importance in comprehensively understanding vector biodistribution and transduction patterns.

Conclusions

The role of 3D CFT in comprehensive biodistribution analysis is instrumental in providing insights into vector biodistribution, tissue-specific transgene expression, and drug development, thereby contributing to advancements in preclinical research and therapeutic strategies. This is supported in the recent publication by REGENXBIO, Inc., where both AAV7 and AAV9 displayed unexpected transgene expressions in facial bone structures and sinus tissues.

The versatility of CFT in applications, including gene & cell therapy, oncology & immunotherapy, and neurology, emphasize the need for further research and development in advanced imaging modalities. The continued exploration of CFT and its integration into preclinical studies holds potential for paving the way for improvements in therapeutic interventions and drug delivery systems.

Reference: 1. Yost SA, Firlar E, Glenn JD, Carroll HB, Foltz S, Giles AR, Egley JM, Firnberg E, Cho S, Nguyen T, Henry WM, Janczura KJ, Bruder J, Liu Y, Danos O, Karumuthil-Melethil S, Pannem S, Yost V, Engelson Y, Kaelber JT, Dimant H, Smith JB, Mercer AC.2023.Characterization and biodistribution of under-employed gene therapy vector AAV7. J Virol97:e01163-23.https://doi.org/10.1128/jvi.01163-23