Quantitative Analysis of Subretinal AAV-GFP in Fluorescent Fundoscopy Images from Non-Human Primates

Judith A. Newmark, Adrian M. Timmers, Chunjuan Song, Guo-Jie Ye, and Mark S. Shearman

AGTC, Cambridge, MA and Alachua, FL

BACKGROUND

Design and optimization of therapeutic vector constructs, including capsid serotypes and promoters, typically evaluates vector efficacy via reporter genes such as GFP and mCherry. Ocular vector constructs are frequently gauged based on fluorescence fundus images (in-life) and immunohistochemistry (termination).

Evaluation of reporter gene expression at different timepoints and in different eyes using fluorescence fundus images requires reproducible image capture settings. Variables known to affect fluorescence intensity in images include instrument, exposure, gain, angle, focal plane, bleb geometry, eye geometry, level of mydriasis, intrinsic autofluorescence, and pattern of fluorescent reporter gene expression. Many of these variables can not be controllably reproduced between imaging sessions.

We developed a quantitative method that normalized the GFP signal in the subretinal bleb to background (fields lacking reporter gene expression, outside the bleb) within each fundus image. This enabled a quantitative comparison of GFP expression among different vector constructs.

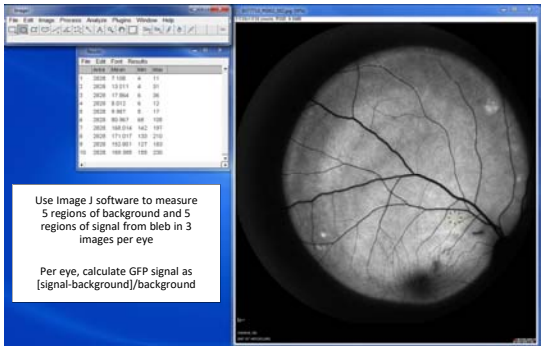
METHODS

Three common serotypes of AAV (AAV5, AAV8 and AAV2tYF) that are currently used in ocular clinical trials were manufactured with a photoreceptor specific promoter and a hGFP reporter gene using triple transfection technology.

Twelve (12) Cynomolgus monkeys (*Macaca fascicularis*) underwent bilateral subretinal injection of $4.5x10^{10}$ vg/eye in 100 µL.

Fundus fluorescence images were collected at pre-dose and 2, 4, 6, 10, and 13 weeks post-dose using a Heidelberg cSLO SPECTRALIS® instrument. Settings for each image were adjusted to avoid overexposure with focus on constant features such as retinal blood vessels. A minimum of 2 images were collected per eye, one of the posterior pole (includes the optic nerve and fovea) and one of the bleb. Additional images to capture the borders of the bleb and retinal tissue outside of the bleb area were collected.

Quantitation was performed using Image J software. A circular polygon region was used to sample the average GFP fluorescence intensity of regions outside (background) and within the subretinal bleb. Per image, the average background fluorescence was subtracted from the average bleb intensity, and then a ratio of signal to background was calculated.

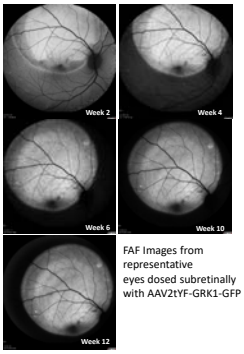


RESULTS

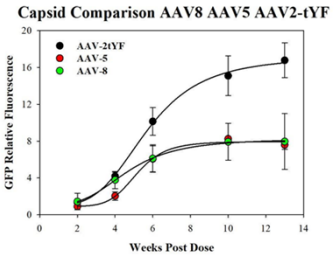
At 2 weeks after injection, the GFP fluorescence signal remained close to background level for all three AAV capsid serotypes. At 4 weeks after injection all three serotypes had GFP signals above background, and an apparent difference in the rate of increase of expression began to emerge. Peak increase in GFP signal occurred between 4 and 6 weeks and was followed by a leveling off after 10 weeks for AAV5 and AAV8. GFP expression continued to increase throughout the study for AAV2tYF.

RESULTS (CONTINUED)

GFP Reporter Fluorescence Analysis

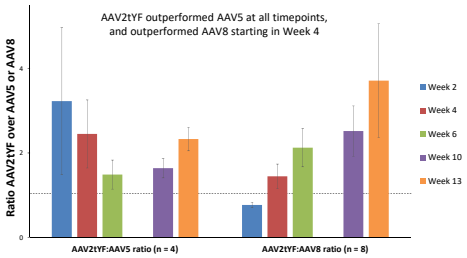


FAF Images from representative eyes dosed subretinally with AAV2tYF-GRK1-GFP

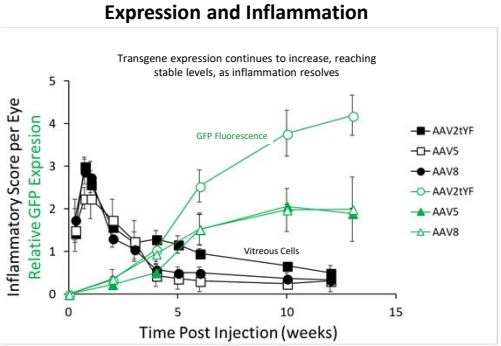


Curve fitting of GFP fluorescence in NHP eyes dosed with AAV2tYF-GRK1-GFP, AAV5-GRK1-GFP or AAV8-GRK1-GFP (4.5×10^{10} vg/0.1 mL; subretinal).

Pairwise ratio of AAV2tYF over AAV5 or AAV8



Expression and Inflammation



CONCLUSIONS

We developed a quantitative method for comparative analysis of subretinal AAV-GFP in fluorescence fundus images that revealed apparent differences in onset and rate of expression among three commonly used capsid serotypes. Differences were detected between images that appeared similar to an observer performing a qualitative assessment. Although this method was developed using GFP expression in non-human primate eyes after subretinal injections, the same method could be extended for use with other fluorescent labels, images from other species or injection through other routes. We are exploring similar methods to analyze GFP expression following intravitreal dosing.

FUTURE PLANS

- Quantitation of total fluorescence across entire subretinal bleb
- Quantitation of fluorescence in images from intravitreal dosing

agtc

Visionary science for life changing cures.