Successful repeated hepatic gene delivery in non-human primates achieved with AAV5 by use of immune adsorption.

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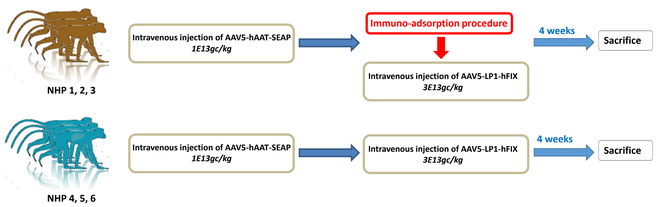
Introduction

A major challenge in AAV-based gene therapy is the presence of circulating neutralizing antibodies (NABs) against AAV vector capsids that may prevent successful transduction of the target cells. NABs can be present in patient's blood prior to AAV treatment due to naturally acquired infections with the wild type AAV virus (pre-existing NABs). Anti-AAV NABs are also raised after first administration of AAV in the course of gene transfer treatment. There is a need to develop strategies that would permit AAV-mediated gene delivery to patients with pre-existing anti-AAV NABs or patients previously treated with AAV-mediated gene transfer in which the therapeutic protein expression has decreased over time due to the natural turnover of transduced cells or insufficient transduction.

Goal / Experimental Design

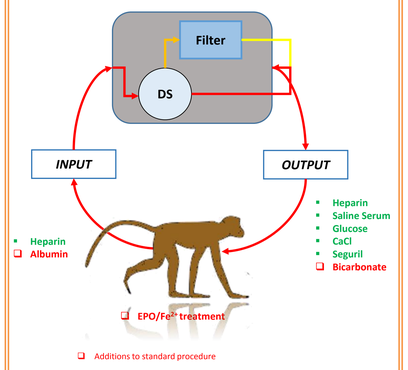
To address those issues, we explored the feasibility of using an extracorporeal immune-adsorption (IAd) procedure for repeated, liver-targeted gene delivery in non-human primates (NHPs). NHPs (3 animals per group, 2 groups) tested negative for the presence of anti-AAV serotype 5 NABs, were first injected intravenously (dose of $1x10^{13}$gc/kg) with AAV5-hSEAP. Four months after the first AAV injection, all the animals were re-injected with AAV5-hFIX (dose of $3x10^{13}$gc/kg). Three of the animals were submitted before the second administration to IAd.



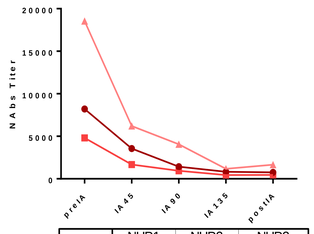
Results

The levels of anti-AAV5 neutralizing antibodies (NABs) were determined immediately before and during the procedure. After 1 session (3 cycles) of immune-adsorption, the levels of NABs were decreased by a mean factor of 11.3. The transduction efficacy of both AAV5-hSEAP and AAV5-hFIX were assessed by measuring the levels of circulating hSEAP and hFIX proteins as well as the amounts of AAV vector DNA present in the liver at sacrifice (four weeks after the second AAV injection). Sequential AAV-based gene delivery with AAV5 after immune adsorption proved to be successful an effective transduction was achieved for the two reporter transgenes (hSEAP and hFIX) used in the study. Furthermore, the hFIX levels obtained after re-administration were in the same range of the hFIX levels obtained after a single primary administration of AAV5-hFIX. In contrast, the re-administration of AAV5-hSEAP followed by AAV5-hFIX without immune adsorption was unsuccessful due to the total inhibition of secondary AAV5 transduction by anti-AAV5 NABs.

1. Implemented IAd procedure



2. NABs fold decrease during IAd

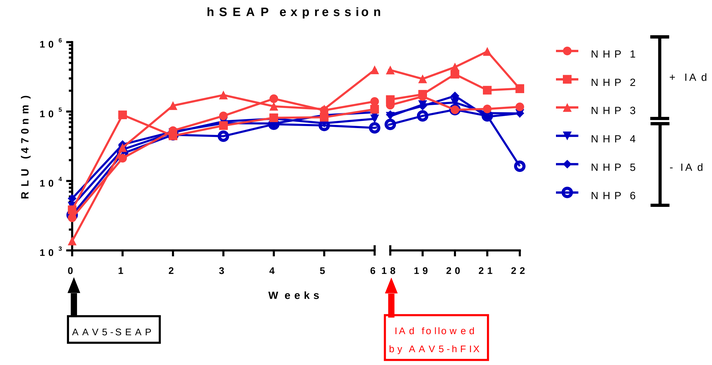


	NHP1	NHP2	NHP3
preIA	8205.64	4812.56	18537.35
IA45	3550.57	1667.16	6201.13
IA90	1432.64	941.82	4060.59
IA135	829.23	448.72	1174.72
postIA	760.75	461.84	1659.86

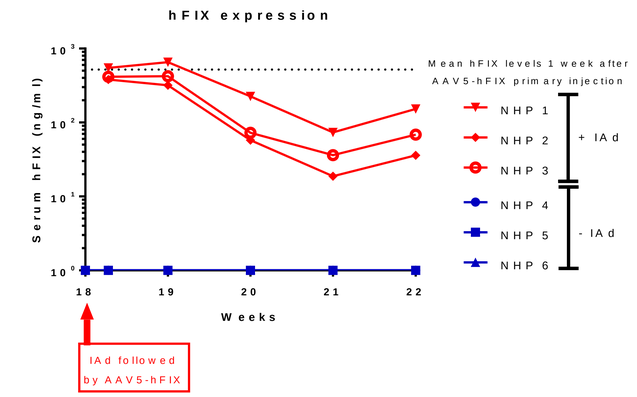
10.3x 10.6x 13.1x

3. Dual transgene expression overtime (hSEAP (a) and hFIX (b)) in NHPs submitted to IAd (red) or not (blue) before AAV5-hFIX administration

a

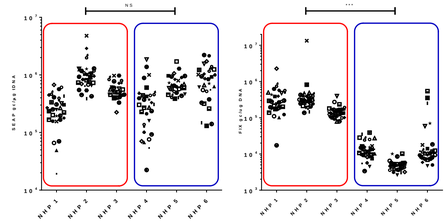


b

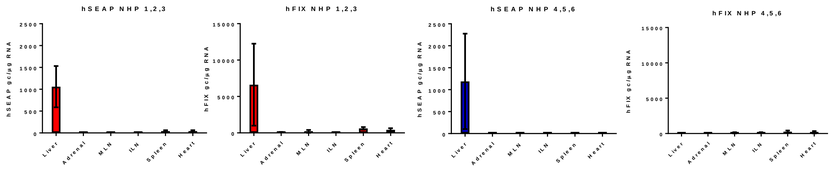


4. (a) AAV5-hSEAP and AAV5-hFIX vector DNA copies in different liver regions ; (b) Biodistribution of transgenes mRNAs

a



b



Conclusion

In summary, our data demonstrates that the use of an immune adsorption procedure enables successful re-administration of an AAV5-based gene transfer in NHPs.

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