GENE THERAPY OF HEMOPHILIA A: IDENTIFICATION OF THE MOST EFFICIENT AAV SEROTYPE VECTOR FOR TRANSDUCTION OF HUMAN LIVER SINUSOIDAL ENDOTHELIAL CELLS

Key Points:

1. A number of gene therapy trials for hemophilia A have been performed with different AAV serotype vectors (AAV5, AAV6, AAV8), none of which are truly human liver-tropic, and therefore proven to be less than optimal.
2. In each of the trials, primary human hepatocytes were targeted, rather than primary human liver sinusoidal endothelial cell (LSECs), which express the human clotting factor VIII (hFVIII), and therefore, forced expression of hFVIII in hepatocytes leads to sub-optimal expression.
3. AAV3 serotype vectors transduce both a human LSEC cell line and primary human LSECs significantly more efficiently than AAV5 and AAV6 serotype vectors, and therefore, should prove to be safe and efficacious in the potential gene therapy in patients with hemophilia A.

INTRODUCTION

Gene therapy of hemophilia A has been performed with several AAV serotype vectors, but none has proven to be optimal. For example, although a BioMarin-sponsored clinical trial with AAV5 vectors at a high dose of $6x10^{13}$ vg/kg led to ~100-200% levels of expression of human clotting factor VIII (hFVIII), over a three-year follow up period, the hFVIII levels declined to ~8% (Pasi *et al*., *N Eng J Med*, 382: 29-40, 2020). Nonetheless, given the overall improvement in disease severity (such as multi-year substantial deduction in annual bleeding rates), this drug was approved by the FDA in 2023 as Roctavian, priced at $2.9M/dose. In a Takeda-sponsored clinical trial with AAV8 vectors at a dose of $3x10^{13}$ vg/kg, all patients lost expression of hFVIII within 1 year (Chapin *et al*., *Haemophilia*, 31: 108-117, 2024). More recently, despite encouraging results from two Phase I/II clinical trials, one with AAV-LK03 vectors (George *et al., N Engl J Med*., 385: 1961-1973, 2021) and one with AAV6 vectors (Leavitt *et al*., *Blood*, 143: 796-806, 2024), two Phase III hemophilia A clinical trials sponsored respectively by Spark-Roche with AAV-LK03 vectors, and Sangamo-Pfizer with AAV6 vectors, were halted. Although some patients treated with Roctavian or other vectors have maintained FVIII levels in the normal range, it has generally been difficult to achieve lasting high levels of FVIII expression (i.e. near or within the normal range). While all these vectors target hepatocytes for transgene expression, FVIII is normally made in liver sinusoidal endothelial cells (LSECs), which are able to efficiently secrete FVIII by co-expression of von Willebrand factor. None of the aforementioned AAV capsids have been shown to efficiently target human LSECs. In our present studies, we evaluated the transduction efficiencies of AAV5, AAV6, and AAV3 vectors in human hepatic cells as well as in human LSECs under identical conditions. These results document that AAV6 vectors are ~623x more efficient than AAV5 vectors, and AAV3 vectors are ~2,600x more efficient than AAV5 vectors in human hepatic cells. Interestingly, AAV3 vectors also transduce human LSECs most efficiently, ~7x more efficiently than AAV6 vectors, and ~51x more efficiently than AAV5 vectors. Taken together, these results suggest that AAV3 vectors may prove to be efficacious as well as cost-effective in gene therapy for hemophilia A by directing FVIII expression to the natural site of synthesis, thereby improving secretion and avoiding mechanisms that downregulate expression as a result of intracellular FVIII accumulation.

RESULTS

First generation of AAV3 vectors are significantly more efficient than the first generation AAV5 (Roctavian) and AAV6 vectors in transducing both human hepatic and liver sinusoidal endothelial cells

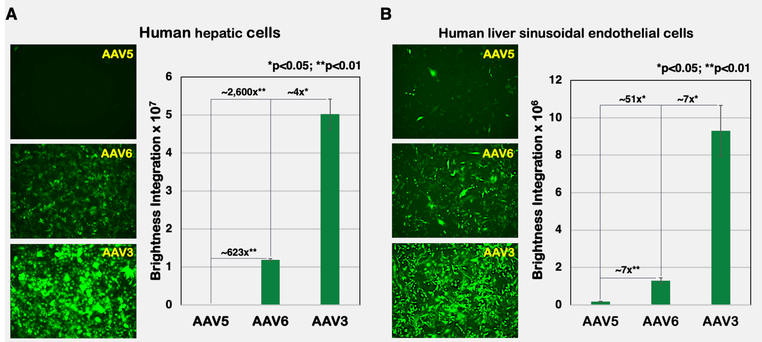

Figure 1: Transduction efficiencies of AAV5-, AAV6-, and AAV3-EGFP vectors in human Huh7 hepatic **(A)** and human SK-Hep1 liver sinusoidal endothelial cells **(B)**. Cells were transduced in triplicates with each vector at $3x10^3$ vgs/cell. Transgene expression was determined 72 hrs post-transduction and quantitated using a Keyence microscope.

First generation of AAV3 vectors are also significantly more efficient than the first AAV5 and AAV6 vectors in transducing primary liver sinusoidal endothelial cells

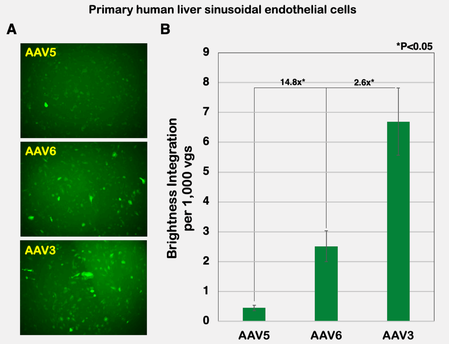

Figure 2: (A) Transduction efficiencies of AAV5-, AAV6-, and AAV3-EGFP vectors in primary human human LSEC cells **(A)**. Cells were transduced in triplicates with each vector at $3x10^4$ vgs/cell. Transgene expression was determined 72 hrs post-transduction. **(B)** Quantitation of the data were performed using a Keyence microscope.

CONCLUSIONS

- First generation of AAV3 serotype vectors are significantly more efficient than other serotype vectors (AAV5, AAV6, AAV8), which have been used to target human hepatocytes in clinical trials for gene therapy of hemophilia A, in transducing human liver sinusoidal endothelial cells (LSECs), the site of expression of human clotting factor VIII (hFVIII).
- Capsid-modified S663V+T492V NextGen AAV3-hFVIII vectors should prove to be safe and effective for long-term gene therapy of hemophilia A.

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