425. Formulation Development for AAV2 Vectors: Identification of Excipients That Inhibit Vector Aggregation

J. Fraser Wright, Tannie Le, Hoc Nguyen, Jurg M. Sommer, Glenn Pierce, Guang Qu.
[1]*Avigen Inc., Alameda, CA.*

Successful development of AAV-based vectors for therapeutic use in humans requires the development of vector formulations that ensure optimal *in vivo* efficacy and safety. Prevention of vector particle aggregation is a critical challenge for parenteral formulation development. The objective of this study was to investigate the mechanism of AAV2 vector aggregation, and to identify classes of excipients to prevent the phenomenon. Previous studies have shown that aggregation of AAV vectors can occur following freeze-thaw cycling and during concentration of purified rAAV (Huang et al, *Mol Therapy* **1**:S286). We have observd that vector aggregation can also be induced following reduction of the ionic strength ($_{\mu}$) by dilution of buffered solutions containing rAAV, and have used this as a model system to investigate the ability of various excipients to inhibit aggregation. Studies were performed on three independent AAV2 vector preparations, one purified by column chromatography (containing empty capsids), a second by CsCl gradient centrifugation (no empty capsids), the third by a hybrid method utilizing both purification process steps (no empty capsids). Dynamic light scattering (DLS) was used to assess aggregation. The contribution of hydrophobic interactions to aggregation was investigated by including Polysorbate 80, Polysorbate 20, or Pluronic F68 (concentrations from 0.01-10%) to inhibit interparticle hydrophobic interactions. At the concentrations examined, these surfactants did not reduced aggregation relative to buffer-only controls in the dilution model. We also examined the effect of glycerol, sorbitol, mannitol, or polyethyleneglycol (0.1-5%), and similarly observed no reduction of aggregation. Based on our previous report that AAV2 aggregation is pH dependent, suggesting involvement of charged amino acids on the vector particle surface (Qu et al, *Mol Therapy* **7**:S348), we examined the ability of charged amino acids (Lys, Arg, Asp, Glu) to reduce aggregation. These amino acids inhibited aggregation only to the degree observed with an equivalent molar concentration of NaCl. The exponential dependence of ionic strength on the valency of contributing ionic species ($\mu=1/2\ \sum c_i z_i^2$) led us to investigate ionic excipients with valency >1. We observed that a number of these excipients inhibited aggregation more efficiently than NaCl. For example, the divalent salt Mg^{2+} SO_4^{2-} inhibited aggregation at a molar concentration approximately 4-fold lower than that required using NaCl. Cumulatively, these data confirm that ionic interactions play a major role in AAV2 vector aggregation, and that use of excipients that maximize ionic strength is a promising strategy to reduce aggregation potential, providing optimal long-term stability and clinical dosing potential for AAV vectors.

426. Novel Chromatographic Process for the Purification of Recombinant Adeno-Associated Virus Pseudotypes

Michael Moxley, Donald Fisher, Anthony P. Green, Timothy D. Kierstead.
[1]*Puresyn, Inc., Malvern, PA.*

Recombinant Adeno-Associated Virus (AAV) is an important vector for efficient gene transfer. AAV generates minimal immune responses and recombination of surface capsid proteins to generate pseudotypes has allowed for the development of more efficient tissue targeting. However, purification processes applicable to multiple AAV pseudotypes presents a challenge and is only now being addressed. Conventional gradient methods using CsCl are cumbersome, difficult to scale and will not meet the anticipated needs for pre-clinical and clinical trials. First generation chromatographic processes are typically based on anion-exchange chromatography and also have limitations.

We report the development of a novel tandem chromatographic process that incorporates anion-exchange and PolyFlo® resins to purify AAV pseudotypes 2/1 and 2/5. The purity of the product exceeds the same vector purified by 2X CsCl gradients as determined by SDS-PAGE (silver stain), Western blot and GC:ICA ratio. The process is reproducible and scalable. Purification of vector from a (50)-150cm^2 plate preparation yields an average of 10^{13} total GC with a GC:ICA ratio of ~200:1. The process has been scaled to 10^{14} input GC and the process uses standard low-pressure chromatography equipment. The process and results have been verified via multiple purification runs for AAV pseudotypes 2/1 and 2/5.

427. Adipose Tissue as a Novel Target for In Vivo Gene Transfer Using Adeno-Associate Virus (AAV) Vectors

Hiroaki Mizukami,[1] Jun Mimuro,[2] Tsuyoshi Ogura,[1] Takashi Okada,[1] Akihiro Kume,[1] Yoichi Sakata,[2] Keiya Ozawa.[1]
[1]*Division of Genetic Therapeutics, Jichi Medical School, Center for Molecular Medicine, Minamikawachi-machi, Tochigi, Japan;* [2]*Division of Cell and Molecular Medicine, Jichi Medical School, Center for Molecular Medicine, Minamikawachi-machi, Tochigi, Japan.*

Currently, skeletal muscle and liver are the preferred target of gene transfer to supply transgene product into systemic circulation. Adipose tissue holds a number of attractive features as a target of gene transfer; it is one of the most abundant tissue in the body, and are designed to secrete various proteins physiologically, making it suitable to supply transgene product into systemic circulation. Of note, the transduced tissue can be safely removed without any sequelae in case of unexpected events. However, efficient transduction of adipose tissue has not been feasible by conventional methods. In order to develop a practical method, we tested the enhancement effect of the excipients upon gene transfer. At first, AAV vectors encoding LacZ were administered into adipose tissue of Db/Db mice. Widespread β-Gal expression was observed when a bio-compatible surfactant was included in the vector solution. To validate the efficacy of this method, vectors encoding mouse erythropoietin (Epo) were utilized. Two weeks following vector injection, significant concentrations of plasma Epo were observed and the levels were almost comparable to that of muscle- or liver-mediated gene transfer. Plasma Epo concentrations kept similar levels during the observation period thereafter. Increased blood hemoglobin levels warrant biological activity of adipocyte-derived Epo. Efficient expression was also confirmed by both immunofluorescence staining of the adipose tissue using anti-Epo antibody and RT-PCR. In addition, following complete removal of transduced adipose tissue, increased plasma Epo concentrations returned normal, suggesting that other tissues were not transduced by this method. Although the precise mechanisms of this phenomenon are yet to be analyzed, our method leads to efficient gene transfer into adipocytes, adding a novel choice for the supplemental gene therapy approaches.