Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

# Intramuscular administration of AAV overcomes pre-existing neutralizing antibodies in rhesus macaques

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ARTICLE INFO

*Article history:*
Received 17 November 2015
Received in revised form 19 October 2016
Accepted 21 October 2016
Available online xxxx

*Keywords:*
Vector
Antibody
AAV
Muscle

ABSTRACT

The seroprevalence of neutralizing antibodies (NAbs) to adeno-associated viral (AAV) vector capsids may preclude a percentage of the population from receiving gene therapy, particularly following systemic vector administration. We hypothesized that the use of intramuscular (IM) administration of AAV vectors might circumvent this issue. IM injections were used to administer AAV8 vectors expressing either secreted or non-secreted transgenes into mice and the influence of NAbs supplied by pre-administration of pooled human IgG on transgene expression was evaluated. We then studied the impact of naturally occurring pre-existing AAV8 NAbs on expression of a secreted transgene following IM vector delivery in rhesus macaques. Finally, we evaluated the ability to readminister AAV vectors via IM injections in rhesus macaques. In mice, the presence of AAV8 NAbs had no effect on gene expression in the injected skeletal muscle. However, liver transgene expression following hepatic distribution of the vector was ablated. In rhesus macaques, naturally occurring pre-existing AAV8 NAb titers of $\leqslant$1:160 had no effect on expression levels of a secreted transgene after IM delivery of the vector. Additionally, readministration of AAV vectors was possible by IM injection into the previously injected muscle groups, with no effect on transgene expression by the original vector. Therefore, the presence of pre-existing NAbs in the human population should not preclude subjects from receiving gene therapy by IM administration of the vector so long as sufficient levels of secreted transgene expression can be produced without the involvement of liver.



## 1. Introduction

Gene therapy has been heralded as the future of medicine for many genetic diseases, and with the approval of the adeno-associated viral (AAV) vector Glybera® for the treatment of lipoprotein lipase deficiency; it is now becoming a reality. A limitation to the effective use of gene therapy is the prevalence of pre-existing neutralizing antibodies (NAbs) to AAV capsids in humans. Currently, early phase clinical trials of systemically delivered vectors are precluding participation of subjects with high pre-existing NAbs.

The impact of pre-existing NAbs potentially could be minimized by using less commonly recognized AAV serotypes, such as AAV8, to which there are less pre-existing NAbs in humans compared to other serotypes [1,2]. In addition, the route of vector administration clearly influences the impact of NAbs. Following intravenous (IV) administration of vector, NAbs towards the vector capsid significantly influence transgene expression, where NAb titers of >1:10 preclude an individual from receiving IV gene therapy [3]. In contrast, pre-existing NAbs have been shown to have less impact on gene transfer following intramuscular (IM) delivery [4–6]. For example, in a Phase I clinical trial for alpha-1-antitrypsin (AAT) deficiency two subjects had pre-existing NAbs to the vector that was used for gene delivery, AAV1 (NAb titers of 1:80 and 1:160). These subjects produced similar AAT levels as those who were sero-negative prior to IM vector injection [4,5]. Therefore, IM

*Abbreviations:* AAV, adeno-associated viral; AAT, alpha-1-antitrypsin; CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; ELISA, enzyme-linked immunosorbent assay; ffLuc, firefly luciferase; GC, genome copies; HIV, human immunodeficiency virus; IA, immunoadhesin; IM, intramuscular; IACUC, Institutional Animal Care and Use Committee; IV, intravenous; IVIG, intravenous immunoglobulin; KO, knockout; mAb, monoclonal antibody; NAbs, neutralizing antibodies; NHP, non-human primate; RAG, recombinase activation gene; SIV, simian immunodeficiency virus; SHIV, simian-human immunodeficiency virus; tMCK, truncated muscle creatine kinase.

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http://dx.doi.org/10.1016/j.vaccine.2016.10.053
0264-410X/

administration of an AAV vector is less susceptible to pre-existing NAbs.

As traditional vaccine strategies have not succeeded in the prevention of human immunodeficiency virus (HIV) infection, passive immunization using viral vectors expressing broadly NAbs to HIV has been developed as an alternative therapy approach [7,8]. This strategy removes the need for repeated parenteral delivery of NAbs as a traditional biotherapeutic. Studies have shown that IM administration of $1.3 \times 10^{10}$ genome copies (GC) to humanized mice (a dose of approximately $3 \times 10^{11}$ GC/kg) resulted in serum expression levels of an immunoadhesin (IA) version of the broadly NAb to HIV, VRC01, at 8.3 μg/ml. This was sufficient to provide protection against subsequent challenge with HIV [8,9], highlighting the potential of this approach.

As other strategies have been successfully used to administer and readminister AAV vectors locally [10], and as there is less influence by AAV NAbs following IM injection, we predict that readministration by IM injection will be possible. The ability to readminister vector will enable a variety of scenarios, including increasing transgene expression by injection of a second dose or expanding the number of HIV NAbs expressed to prevent viral escape. In this study, we investigated the influence of AAV NAbs on transgene expression following IM administration of AAV8, first in mice and then in non-human primates (NHPs).

## 2. Materials and methods

### 2.1. AAV vector production

AAV vectors were produced by the Penn Vector Core at the University of Pennsylvania as previously described [11].

### 2.2. Mice

Male C57BL/6 and recombinase activation gene (RAG) knockout (KO) mice 6–8 weeks of age were purchased from Charles River Laboratories (Wilmington, MA, USA) and The Jackson Laboratory (Bar Harbor, ME, USA), respectively. Mice were housed under pathogen-free conditions at the University of Pennsylvania's Translational Research Laboratories. Animal procedures and protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania. IM vector administration and passive transfer experiments were performed as described previously [12,13]. Pooled human IgG (intravenous immunoglobulin [IVIG]) was purchased from Carimune NF (CSL Behring, Bern, Switzerland).

### 2.3. Non-human primates

Rhesus macaques (Chinese origin, captive bred, 2.8–7.7 kg) were housed at the NHP Research Program Facility. Studies were performed according to a study protocol approved by the Environmental Health and Radiation Safety Office, Institutional Biosafety Committee, and IACUC of the University of Pennsylvania. Studies in African green (USA born, captive bred, 1.8–2.4 kg) and squirrel monkeys (Bolivian origin, captive bred, 0.7–0.8 kg) were conducted at Covance (Princeton, NJ, USA) in accordance with their IACUC. IM vector administration was performed as described previously [12].

### 2.4. NAb titration assay

NAb assays were performed on serum as described previously [2].

### 2.5. Detection of secreted proteins

AAT and antibody transgene expression were measured in serum from mice and NHPs using an enzyme-linked immunosorbent assay (ELISA) as described previously [1,12]. For NHP samples, ELISA plates were coated with gp120 proteins from strains of simian-human immunodeficiency virus (SHIV) or simian immunodeficiency virus (SIV) to which the antibodies bind diluted in phosphate-buffered saline (PBS, Immune Technology Corp., New York, NY, USA).

### 2.6. Statistical analysis

All analyses were performed in Prism (GraphPad Software, San Diego, CA, USA) using either an unpaired Student's *t*-test or analysis of variance for significance (Tukey's or Dunnett's multiple comparisons test).

## 3. Results

### 3.1. Pre-existing vector immunity ablates liver gene expression

To create a model of gene expression in the presence of pre-existing vector immunity, passive transfer experiments were conducted. C57BL/6 mice were administered IV with 200 μl of pooled human IgG (intravenous immunoglobulin [IVIG]) or naïve mouse serum two hours prior to AAV vector injection. IVIG contained NAbs to AAV8 of 1:320 [2]. Mice were then administered with $10^{10}$ GC of an AAV8 vector expressing firefly luciferase (ffLuc) driven by a modified chicken beta-actin promoter (AAV8.CB7.ffLuc) into the right gastrocnemius muscle. On day 7, mice were imaged to quantify ffLuc expression.

In mice pre-administered with naïve mouse serum (denoted as 0 mg IVIG), IM administration of AAV8 resulted in highly transduced skeletal muscle with substantial hepatic targeting (Fig. 1A). Quantification of ffLuc revealed high transgene expression in muscle and liver, although the absolute expression of ffLuc could not be compared as the efficiency of imaging in these two organs differs (Fig. 1C). The proportion of liver gene expression following IM vector administration was greater than we reported previously [12], likely due to greater hepatic activity of the CB7 promoter compared to the previously used cytomegalovirus (CMV) promoter.

IM administration of $10^{10}$ GC of vector in the presence of 12 mg IVIG completely ablated liver ffLuc expression (Fig. 1B). Dose-titration of IVIG revealed a threshold level of pre-existing NAbs above which hepatic gene expression was ablated as pre-administration of 1.2 mg IVIG produced a 150-fold reduction in liver ffLuc expression and higher doses abrogated expression to background levels (Fig. 1C). In contrast, muscle gene expression was not affected by any dose of IVIG studied. The influence of IVIG on gene expression in skeletal muscle was vector dose-dependent as liver gene expression was significantly reduced at 1.2 mg IVIG with a concomitant decrease in muscle expression when the vector dose was decreased ten-fold (Supplementary Fig. 1). At the lower vector dose, transgene expression was completely ablated in muscle and liver at 40 mg IVIG. Thus, the ability to express an AAV gene therapy product in the presence of pre-existing vector immunity is influenced by both the level of NAbs and the AAV vector dose injected.

### 3.2. Pre-existing vector immunity reduces secreted protein expression in mice

As the presence of pre-existing AAV NAbs significantly affected liver gene expression of a non-secreted protein (ffLuc) following IM

Please cite this article in press as: Greig JA et al. Intramuscular administration of AAV overcomes pre-existing neutralizing antibodies in rhesus macaques. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.10.053