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A seroprevalence study of the presence of Adenovirus Associated Virus Vector serotype AAV5 neutralizing activity and antibodies in Patients with Haemophilia A

Stanford S.N., [1] Oyesiku L., [1] Pink R., [1] Creagh D., [2] Clark A., [3] Wilde J., [4] Lowe G., [4] Curry N., [5] Pasi J., [6] Needham J.[1] and Rangarajan S. [1]

[1] Department of Haemophilia, Haemostasis and Thrombosis, Hampshire Hospitals NHS Foundation Trust, Basingstoke, Hampshire, UK [2]Haematology Department, Royal Cornwall Hospital, Truro, Cornwall, UK [3]Bristol Haemophilia Centre, Bristol, UK, [4] Haematology Department, University Hospitals Birmingham Foundation Trust, Birmingham, UK, [5]Haematology Department, Oxford University Hospitals NHS Foundation Trust, Oxford, UK, [6] Haematology Department, Barts Health NHS Trust, London, UK.

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INTRODUCTION

The main stay treatment for Haemophilia A is the replacement of congenitally deficient factor VIII (fVIII) through plasma-derived or recombinant concentrates (1). Although replacement of fVIII has improved life expectancy and quality by reducing joint damage and spontaneous bleeding there are limitations including frequent infusions and high costs (2). Gene therapy is a potential alternative treatment which is currently undergoing phase I clinical trials. This form of therapy utilises an adeno associated viral (AAV) vector containing the human genetic code for fVIII that targets liver cells. This enables the liver to produce fVIII and even modest increases (plasma level of 2ng/ml induces an increase in activity of 1%) can ameliorate severe forms of the disease (3). However, the presence of antibodies against these AAV serotypes prevents this form of treatment in some patients. A previous study on the presence of neutralizing activities against AAV 2, 5 and 8 in a paediatric haemophilia population found it be 43.5% , 25.8% and 22.8% respectively (4).

AIM

This observational study aimed to establish the seroprevalence of AAV 5 by testing neutralising activity and total antibodies in the adult haemophilia community in the United Kingdom.

HOW IS AAV USED IN GENE THERAPY?

Gene therapy is designed to introduce genetic material into cells to compensate for abnormal genes or to make a beneficial protein (5). Introduction of a normal copy directly into the cell is not beneficial, instead a vector is required to carry the normal copy of the gene into the cell. Modified non-disease causing viruses are used as vectors as they can deliver the new gene by infecting the cell. Retro viruses integrate their genetic material into the chromosome of the cell (6) whereas adenoviruses, introduce their DNA into the nucleus of the cell but the DNA is not integrated into the chromosome (7) (fig 1). Adeno associated viral vectors have been used to date in the treatment of patients with Haemophilia B with successful results (8).

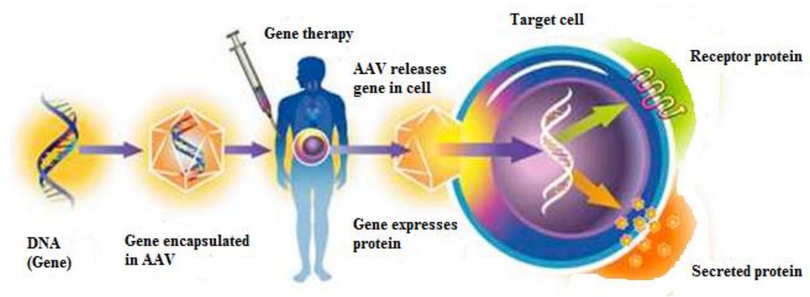

Figure 1: Illustrates the mechanism by which AAV integrates in a host target cell and secretes the required protein. Image adapted from Tamer A. Malik, et al , Beth Israel Deaconess Medical Center (9).

METHODS

Previously diagnosed patients with Haemophilia A identified from seven sites in the UK were invited to take part in the study. Patients who were 18 years or over, previously treated with fVIII concentrates and gave informed consent were included in the study. Those lacking mental capacity to consent or previously not treated with fVIII were excluded from the study. This study was given a favourable ethical opinion by the Liverpool Central Research Ethics Committee (REC 15/NW/0469). Citrated plasma samples from 101 Haemophilia A patients were tested for pre-existing neutralising activities and antibodies against AAV 5 using AAV transduction inhibition activity assay and total antibodies assay. This study was funded by a grant from BioMarin Pharmaceutical Inc.

ASSAY PRINCIPLES

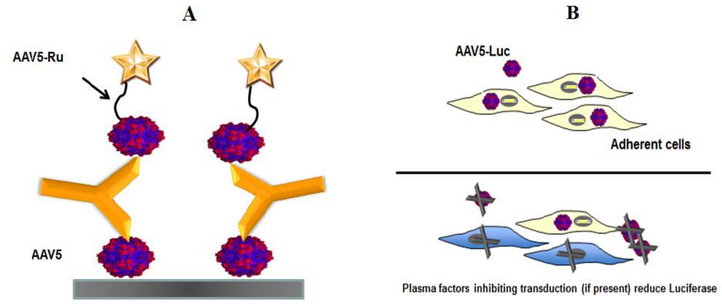

Figure 2 A&B: Illustrates the principle of the total antibody assay (TAb) where binding of the ruthenium labelled AAV5 capsid to AAV5 antibodies causes changes in the emitted light which quantifies specific binding as seen in figure A. Image adapted from http://msg.mbi.ufl.edu/res-aav.html (10). The transduction inhibition (TI) assay measures the plasma's ability, when AAV5 neutralizing factors are present, to neutralise the transduction of 293T/17 cells by a recombinant AAV5 vector carrying the luciferase gene reporter as seen in figure B. The department of Bioanalytical Sciences at BioMarin Pharmaceutical Inc developed the assay methods utilised in this study.

RESULTS & CONCLUSION

Table 1: Demographics of the Haemophilia A population

Baseline Demographics	
	Mean value
Age (years)	42 (20-81)
Weight (kilograms)	80.8 (53.5-128)
Baseline FVIII level	**No. of patients**
<1%	60
1%-5%	14
>5%	26
Exposure to plasma products	**No. of patients**
Yes	86
No	15
HIV status	**No. of patients**
Positive	6
Negative	94
Hep C exposure	**No. of patients**
Yes	40
No	60

Table 2: Seroprevalence of AAV5 in the United Kingdom based on TI and TAb assays.

	Transduction Inhibition Assay	Total Antibody Assay
% positive patients	25	21

Table 3: Comparisons (%) between the TI and TAb assays

Haemophilia A	
TAb- TI -	71%
TAb+ TI+	16%
TAb+ TI-	5%
TAb- TI+	9%

The seroprevalence of AAV in our cohort was similar to that observed in other studies (4) and confirms the presence of neutralising activity and antibodies to AAV5. As there is a difference in the percentage of positive patients between the two assays at this stage both are used to identify patients eligible for gene transfer.

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