Gene therapy in Haemophilia B: a global vision of its success and weaknesses

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Introduction

Haemophilia B is an X-linked bleeding disorder

Cause: mutations in the gene encoding blood coagulation factor IX.
Prevalence: 1/20.000 live male adults worldwide.
Current treatment: intravenous infusion of clotting factor concentrates.
Economic cost: $200.000 per year for an adult with severe disease treated prophylactically.
Classification: severe (<1%), moderate (1-5%) or mild (>5%) according to circulating FIX levels.

It is a good target for gene therapy:

- Monogenic disease
- Wide therapeutic window
- Small and large animal models
- Easy to monitor

Gene therapy pretends to add a correct gene, which will supply the missing protein

Adeno-associated viruses

- Small, non – pathogenic and single-stranded DNA viruses
- Replication – defective: need of a helper virus for replication and completion of their life cycle.
- Genome composed by two genes: *rep* and *cap*.

AAV vectors

- A therapeutic expression cassette replaces *rep* and *cap* genes, leaving the viral ITRs as the only viral sequences.
- Transduction of non-dividing cells, remaining in an episomal form.
- Limited packaging capacity (4700 nucleotides).

Objectives

The purpose of this work is to understand the current situation of gene therapy in Haemophilia B and to assess the upcoming solutions proposed to solve the problems appeared in the clinical trials, after the vector transfer.

Methods

The articles used for the realization of this review were search scientific databases using the following key-words: haemphilia B, gene therapy, AAV vectors, immune response and clinical trials. For the elaboration of the final work, the most relevant information was selected.

Evolution of the therapy

1989 – FIX detected in mice circulation after transplanting fibroblasts transduced *ex vivo* with a retrovirus containing hFIX.

90s – Several studies in mice and dogs using non-viral, retroviral adenoviral and AAV vectors.

2003 – First clinical trial in humans: intramuscular injection of a rAAV vector encoding FIX transgene.

Results - The two main clinical trials

Trial / Year	Pre-clinical studies	Vector and route of administration	Subjects	Results	Problems and side effects	Conclusion
Manno et al, 2006	Studies showing long-lasting expression of FIX levels in dogs injected with a single infusion of rAAV vector either into the portal vein or the hepatic artery.	• rAAV-2 vector containing hFIX gene with a liver-specific promoter (AAT promoter, APOE enhancer and elements of HCR). • Vector delivered through the hepatic artery.	7 subjects divided in 3 dose-cohort: •$8x10^{10}$ vg/kg •$4x10^{11}$ vg/kg •$2x10^{12}$ vg/kg	A subject reached a circulating FIX of 11.8% which persisted during 10 weeks.	• Asymptomatic transaminitis 4 weeks after vector infusion. •Presence of neutralizing antibodies to AAV. • No formation of inhibitory antibodies to the transgene product.	Expression of FIX in liver can be achieved after an AAV2 injection. Confirmation of a T cell response against capsid antigens, not seen in animals , that destroyed transduced hepatocytes.
Nathwani et al, 2011	Safety studies on macaques that maintained a stable level of transgene expression for 5 years after being transduced with a scAAV vector directed to liver.	• scAAV-8 vector containing the PL promoter and a codon-optimized hFIX. •Vector delivered through intravenous infusion.	6 subjects divided in 3 dose-cohort: • $2x10^{11}$ vg/kg • $6x10^{11}$ vg/kg • $2x10^{12}$ vg/kg	Subjects in the low and medium cohort experienced a modest improvement of circulating FIX levels (between 1-3%). Subjects in the high cohort, achieved FIX levels of 7% and 8-12% respectively declining until 3% and 5%, but persisting for 6 months and more than a year.	• Subjects in the high cohort experienced a rise in liver enzymes (asymptomatic transaminitis), reduced after a steroid treatment. • Dose-dependent AAV8 capsid-specific T cell response after gene transfer. • No formation of neutralizing antibodies to the transgene product.	FIX expression after a intravenous infusion remains stable during months and, for one case, the level achieved reaches about 5%, which results enough to convert haemophilia from severe to mild. A larger number of participants will be needed to evaluate the clinical significance of the elevation in aminotransferase levels.

Problems and solutions proposed:

Asymptomatic transaminitis resulting in the destruction of the transduced cells

• In contrast to experimental animals, AAV2 naturally infects humans during childhood together with a helper virus. This causes activation of the innate immune system and forms $CD8^+$ T cells directed to the antigens. After the infection, a small pool of memory T cells are maintained and can be reactivated in a reexposure of AAV capsid, destroying the transduced cells.

• Memory $CD8^+$ T cells are activated though the recognition of capsid-derived peptides presented on the cell surface in MHC class I.

• When hepatocytes are destroyed, cytosolic enzimes are released to the circulation causing transaminitis.

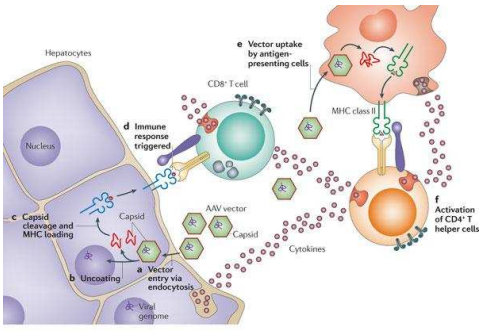

Figure 1 | Explanation of the rise in liver enzymes observed in the clinical trial. Mingozzi FH et al, 2011.

• The transaminitis can be solved administrating a short-term immunosuppressant treatment that blocks the response to capsid antigens until they are completely cleared from the transduced cells.

Formation of antibodies against the transgene product

Some patients (about 3%) develop antibodies against the therapeutic protein. Studies in non-human primates showed an eradication of inhibitory Ab against human FIX after a treatment with cyclosporine A and rituximab (Mingozzi F et al, 2012).

Pre-existing neutralizing antibodies against AAVs

Antibodies against AAVs remain in most of the population, after being infected. This causes a decrease in transduction efficacy which could be solved creating an engineered capsid.
AAV2(Y-F) mutant capsid preserve gene expression and minimize the loss of transduced hepatocytes because:

- There are no pre-existing neutralizing antibodies against the engineered capsid.
- The specific mutation causes a decrease in proteasome-mediated degradation of AAV2 vectors.

Zhong Li et al, 2012

Increasing the efficacy of gene transfer : use of Factor IX Padua

Factor IX (R338L) gene results in a hyperfunctional protein (known as factor Padua), with a clotting activity of eight times the normal level (Simoni P et al, 2009). The use of this mutant gene in the therapeutic cassette could increase the efficacy of gene transfer.

Conclusions

▪ Liver-directed gene transfer using AAV vectors could provide long-term expression of FIX, enable to convert haemophilia B from a severe to a mild form.

▪ Immune response to the transduced cells represents the main barrier. Nevertheless, it could be managed through:

- ⟹ Immunosuppressant treatments
- ⟹ Engineered capsids

▪ Trials enrolling more subjects will be crucial to assess new approaches (e.g. factor IX Padua) and individual variations of the therapy.