GENE THERAPY PROTOCOL DESIGN FOR HEMOPHILIA A

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INTRODUCTION:

Hemophilia A is a monogenic disease caused by mutations in the gene encoding human factor VIII (FVIII), resulting in the inability to properly form a blood clot.

FVIII gene therapy attempts to rectify the presence of a mutant *F8* with the addition of a functional gene.

Hemophilia A is a prime candidate for gene therapy in that only a moderate increase in FVIII activity (2-5%) is required to be therapeutically effective.

PROTEIN GENE CHOICE

To overcome the low transgene expression we use a hybrid human/porcine FVIII molecule (HP-FVIII). This high-expression transgene have the B-domain deleted.

Figure 2. Crystal structure of a truncated B-domain human factor VIII (from PDB)

Figure 1. Schematic representation of the therapeutic protein designed. p means that it is a porcine domain, h means that it is a human domain

VECTOR CHOICE

At present, adenovirus-associated virus (AAV) vectors shows the greatest promise for long-term correction of hemophilia.

AAV8 (serotype 8) have a lower seroprevalence and have the highest level of hepatocyte transduction.

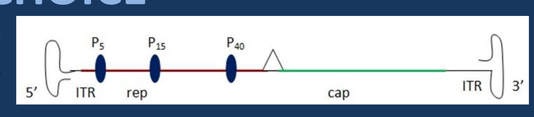
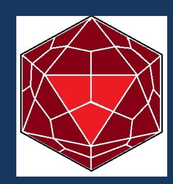


Figure 3. Schematic genome structures of a wild adeno-associated virus.



PROBLEM ⇨ **LIMITED GENOME PACKAGING CAPACITY**

GENE STRUCTURES PRODUCTION

FVIII → ~9000 pb AAV vectors → limited genome packaging capacity of ~5 Kb

To circumvent this problem the transgene was divide into two chains and introduce each in different vectors.

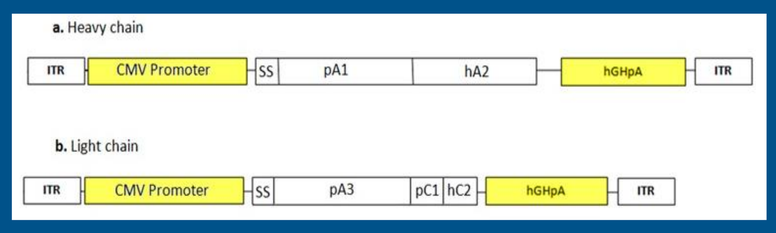


Figure 4. Schematic representation of the gene structures of AAV8. **(a)** HP-FVIII-HC **(b)** HP-FVIII-LC. CMV is the promoter used; hgH pA, human growth hormone polyadenylation signal; ITR, AAV inverted terminal repeat; SS, signal sequence.

VIRAL VECTOR PRODUCTION

This process has to be done twice, one using the heavy chain and other with the light chain.

Thus we get two groups of vectors containing both FVIII chains that we will use in therapy.

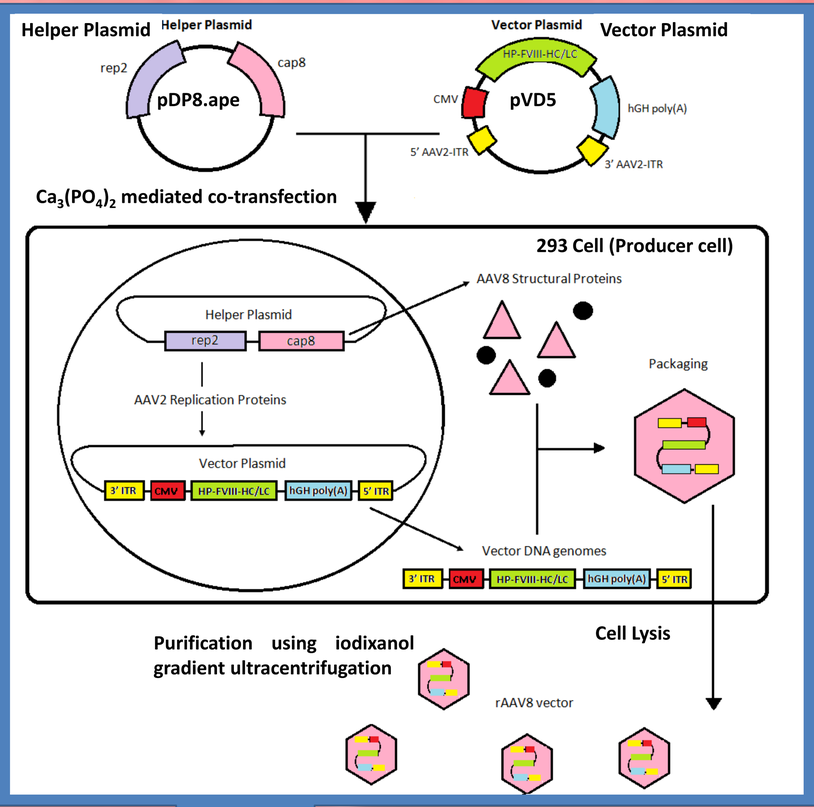
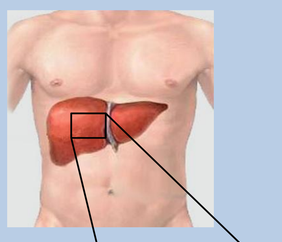
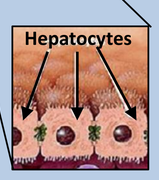
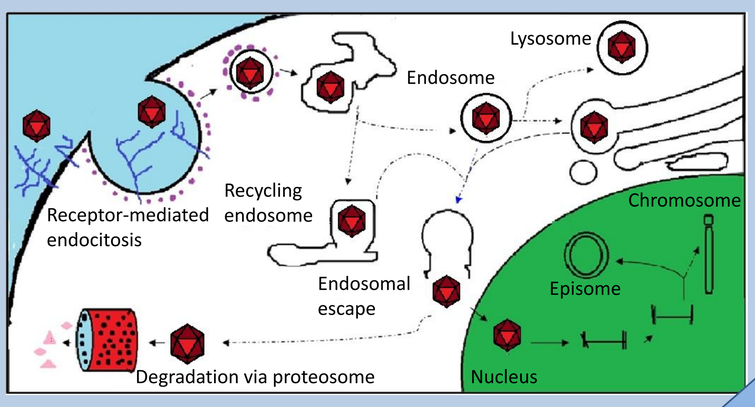


Figure 5. Schematic production of recombinant AAVs serotype 8 using 293 cells.

TRANSDUCTION

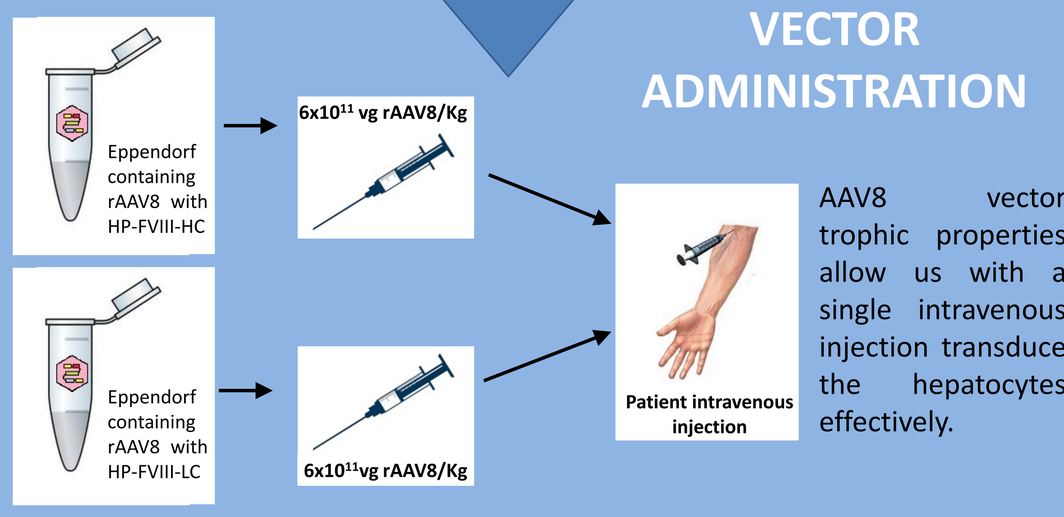






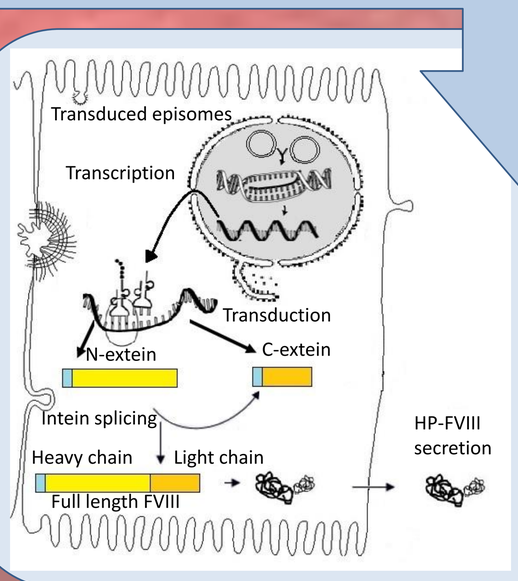
AAV8 transduction efficiency via intravenous injection is very good.
In just 30 minutes, 95% of the vectors injected into the bloodstream will remain in the liver, transducing hepatocytes.

VECTOR ADMINISTRATION



AAV8 vector trophic properties allow us with a single intravenous injection transduce the hepatocytes effectively.

EXPRESSION



According to some studied bibliography the patient dose administered predict a FVIII expression duration over 8-9 months, although duration may vary depending on the patient.

Figure 6. Illustration of the intein mediated dual AAV system for expressing FVIII. Note that splicing reaction occurs in the cytoplasm after polypeptides for FVIII have been translated. Once the protein has been generated, it is secreted by hepatocytes to bloodstream where the clot factor will generate its function.

TOXICITY AND SIDE EFFECT

As we introduce two different vectors to treat patients, the dose is doubled (2x viral vectors in the patient), this may cause severe immune problems in patients.

According to the read articles the only side effect that can have therapy is an aminotransferases increase. The increase makes low aminotransferases expression levels, but treatment with glucocorticoids rapidly reduces aminotransferases levels.

PROTOCOL DISCUSSION

- Effective hemophilia gene transfer requires a sustained, long-term (years) production of coagulation factors at therapeutic levels be generated. Gene therapy aims to improve quality of life of the patients avoiding that have to suffer so many infusions as frequently.
- This protocol has been designed through the study of different papers. The method of gene delivery must be safe, and the risk of immune response must be minimal. This gene transfer approach, which could be effective in humans, will have be tested both in the laboratory and in the clinic. The therapy would improve significantly the hemophiliac patients quality of life.
- Gene therapy continues to hold promise for the permanent correction of hemophilia. Future studies need to address immunological and safety issues.