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Advancements in gene therapy for hemophilia: Overcoming challenges with lentiviral vectors

Summan Aslam

Department of biochemistry and molecular Biology COMSATS University Islamabad, Pakistan.

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A B S T R A C T

Hemophilia is X-linked recessive inherited trait that is resulted from faulty or deficiency of clotting factors VIII and IX (genes) i.e., Hemophilia A (HA) and Hemophilia B (HB) respectively. Absence of these factors causes stoppage of coagulation cascade that results in bleeding problems to internal organs. HA is more prevalent than HB with US bearing the most cases in the world and it has been reported that white is affected more than black people. Conventional treatments didn't give complete relief and need to be repeated throughout life but gene therapy could be the possible and long-lasting treatment that gives hope to treat genetic disorders. Two types of viral vector systems are used to deliver the cargo i.e AAV and LV vectors. The main loopholes of AAV vector are firstly, the capacity of cargo i.e 4.7 kb however the FVIII transgene size is 4.4 kb that restrict choice for promoter and secondly, the gradual loss in transgene expression. These loopholes have been overcome with the use of LV up to greater extant. HIV derived self-inactivating lentivirus vector is used to for the transformation of CD34+ cells. The autologous cell product mechanism resulted in a successful restoration of F8 production diminishing hemophilia A.

Keywords: Hemophilia, Adenoviral vector, Gene therapy

Corresponding Author: Summan Aslam
Email: summanasalam.12@gmail.com

INTRODUCTION

Hemophilia is a group of hemorrhagic-disorders that are inherited as X-linked recessive trait that results in diminishing of or faulty critical factors (clotting factors) that play important role in coagulation-cascade. Bleeding episodes (most specifically to joints) are resulted from compromised-thrombin production and fibrin-clot formation in patients with hemophilia (Peyvandi et al., 2016). Hemophilia is mainly classified by the altered function or deficiency of clotting factors VIII (Hemophilia A) or IX (Hemophilia B) with severity being dependent on the altered clotting-factors activity. Hemophilia A is more prevalent being responsible for more than 80% of cases while Hemophilia B is approximately 5 times less prevalence rate. The disorder mostly affects male and the frequency for Hemophilia A is 1 in 5,000 and Hemophilia B incidence of 1 in 30,000 live male births (Castaman and Matino, 2019). The disorder is more prevalent in White people compared to the Blacks (Soucie et al., 2020). A meta-analysis study arranged for 06 countries which includes Australia, UK, US revealed that the frequency for hemophilia A is 17.1 of 10,000 live male births with US having the most prevalence rate (30,000). Still the prevalence characterizes the disorder as rare according to US definitions (<200,000) cases (Iorio et al., 2019). Symptoms of hemophilia include internal bleeding to joints and brain, unexplained bleeding after injections and cuts, blood in urine, swellings and damage to joints and nose-bleed etc. The basic treatment of hemophilia is the replacement therapy in which concentrates of clotting factors (VIII/IX) are slowly injected into veins. These

concentrates are made from human-blood hence there is possibility of getting infection which has been reduced by replacing blood born concentrates with recombinant concentrates. The main discrepancy in replacement therapy is that it should be done on regular basis (prophylactic therapy) and is also very risky and expensive (Delgado-Flores et al., 2022). Recent advances in gene therapy and gene editing techniques have opened a new era to treat genetic disorders and genomic defects. It is hypothesized and scientists believe that gene therapy for clotting factor genes could be the possible, long lasting and cheaper treatment of hemophilia. Research studies are in progress to find a way to edit and correct the faulty gene to improve production of correct clotting factor in the patient's body. The aim for gene therapy is to produce the clotting factors in the body to avoid the prophylactic replacement therapy and risks of infection (Nienhuis et al., 2017).

DISCUSSION

The causative defected molecules responsible for hemophilia are clotting factors VIII and IX both of which are produced in liver and secreted into the blood-stream. Efficient treatment strategy was observed with injection of recombinant clotting factors but that is only available in highly developed countries because of cost effectiveness and lack of resources. These treatment policies gave an insight to prompt efforts to develop novel treatment-strategies for gene therapy. Advance clinical studies have focused attention on potential gene-therapy to fulfil the needs by showing long lasted benefits of single intravenous injection of an Adeno-associated viral vector (AAV) carrying transgene of functional factor VIII and factor IX to the liver in adult patients of HA and HB respectively (George et al., 2017). However, a decreasing trend was observed in FVIII-transgene expression for an unknown reason but that can be possibly related to stable maintenance of functional episomal-vector genomes reach their packaging limit (Pasi et al., 2020). HIV-derived lentiviral vectors (LV) provide some efficient system to overcome the limitations of AAV based gene therapy. While keeping in mind about the advantageous and discrepancies two different vector systems are used in gene therapy of hemophilia i.e AAV based gene therapy and lentivirus based gene therapy.

Adenoviral Vector Based Gene Therapy

Of all the new techniques emerged for the treatment of hemophilia the problem of curing the disease remain unresolved but it is believed that the gene therapy of liver with single AAV-vectors can be the potential solution and is under clinical-trial stage (Butterfield et al., 2020; Makris, 2020). AAV vectors have developed as the efficient in vivo gene-therapy for HA because of its safety profile and long-time transgene expression from hepatocyte cells (Nathwani et al., 2017). However, there are some challenges like the huge size of the transgene (7kb) of FVIII that exceeds the AAV cargo limit of ~4.7 kb. It is why in clinical trial studies, FVIII product was having deleted B-domain of F8 transgene whose size were approximately 4.4kb. Using such huge size transgene restrict the choice of polA-signals and the promoter sequences because of limiting the space, due to this heterogeneous truncated-genome library is generated as improper packaging occurs (Makris, 2020).

It was observed that the amount of factor VIII in body after transgenesis reduces with the passage of sometime. Schutgens, R. E. recently conducted a study on this question "How Long Will It Last" and reported that AAV-based gene-therapy for HA is successful. According to him in clinical trials of both the BioMarin and the Pfizer decline was observed in the next years however valoctocogene roxaparvovec trials shown the break-even time of eight-years with loss of 5.7% annually (Schutgens, 2022). But the recent BioMarin trials proves this loss as an optimistic and it was found that the decline rate is 44% annually (Ozelo et al., 2022). However, data from the spark-trials give more stable FVIII expression indications.

From the phase 3 trial results it was reported that toxicities in liver, mostly low grade albeit, occur in the beginning or first year in at least some individuals in all cases of both HA and HB trials and that are poorly understood. Furthermore, it was reported that the gradual decline in the transgene expression could be covered by repeat doses but the immune response of body limit this as overexpression causes phenotoxicity (Kaczmarek, 2022).

Gene Therapy of Hemophilia by Lentivirus Vectors

In the last few years, researchers have been working on developing new and more advanced hemophilia therapies. More recently, protein products are in trials for the treatment of hemophilia. In spite of encouraging advancement in protein products such as activating factor 8 mimic antibodies, gene therapy for the disease person with hemophilia remains the best course until now. Adeno associated virus vectors are mostly used in gene therapy due to its success in clinical investigations. Clotting factor FV8 and FV9 expression cassettes are transformed to liver cells where the transgene restores the clotting function of blood effectively. Adeno associated viruses has its limitations, one the formation of episome in the nucleus second, they are administrable to adults but pose a challenge in juvenile

patients, considering the hepatocytes divisions during liver growth in children the episome gets disintegrated resulting in reoccurrence of hemophilia phenotype. Third, the broad preexisting immunity to the adeno associated viruses. Adeno associated virus gene transfer for hemophilia is the most sophisticated but additional hepatocyte base vectors have been developed for gene therapy of hemophilia. In this section we will review HIV derived lentivirus gene transfer (Cantore and Naldini, 2021).

In a recent lentivirus vectors study, conducted in mice, dogs, and non-human primates in which the clotting factor8 transgene is successfully transferred to the experimental models. The coagulation factor8 reached to the therapeutic range in blood of hemophilia A phenotype. Codon optimization of the B domain deleted clotting factor 8 gene resulted in the ten-to-twenty-fold increase in expression in human hepatic cell lines and mice (McIntosh et al., 2013; Ward et al., 2011). The integration of XTEN, an unstructured polypeptide in recombination with LV clotting factor 8 transgene led to an increase in the stability of the protein and increase activity of F8 factor is observed in vivo (Milani et al., 2022). Transgene having both clotting factor 8 and XTEN results in a three to four times increase in half-life of F8 protein when compared to F8 to humans without any adverse effect or evoking an immune response like activating F8 antibodies (Konkle et al., 2020). In the investigation conducted by (Milani et al., 2022) a ten-to-20-fold increase in the activity of transgene F8 was observed when XTEN was inserted in mice and non-human primate models, reducing the LV dose required for therapeutic efficacy for hemophilia. Also reliving the concerns about dose dependent LV toxicity.

Lentivirus vectors associated with autologous hematopoietic stem cells and progenitor cells forms a new therapeutic model for curing hemophilia A. The process contains an autologous CD34+ cells which is transformed with transgene encoding clotting factor 8 responsible for the restoration of hemophilia phenotype. HIV derived self-inactivating lentivirus vector is used to for the transformation of CD34+ cells. The autologous cell product mechanism resulted in a successful restoration of F8 production diminishing hemophilia A (Finkelshtein et al., 2013).

CONCLUSION

Hemophilia is X-linked recessive inherited trait that is resulted from faulty or deficiency of clotting factors VIII and IX i.e Hemophilia A (HA) and Hemophilia B (HB) respectively. Bleeding is the main problem with hemophilic patient i.e bleeding to joints, brains, bleeding from cut that causes serious threats to the life of patient. Replacement therapy, Desmopressin (Human made Hormone) and Emicizumab are some of the conventional treatments for the hemophilia but the basic research from two decades and the recent results from the clinical trials have risen the hope of hemophilic patients towards gene therapy. Mainly two viral vectors i.e Adeno Assosiated Virus vector and the lentiviral vectors are used to deliver the cargo (Factor VIII and FIX) into the hepatocytes. This approach is attractive and give fruitful results but still there are lot of obstacles to achieve the required results. To overcome these obstacles, it needs further research.

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