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“Breaking Paradigms: Towards a Multi-, Inter- and Transdisciplinary Science” In commemoration of the 250th Anniversary of Alexander von Humboldt

REVISION/REVIEW

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Gene therapy in liver diseases focus on Adeno-Associate Virus Vector (AAV) and Virus-Like Particles (VLPS)

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ABSTRACT

The liver has a critical role in several genetic inherited and acquired disorders. Over the years, the development of several therapies to treat liver diseases resulted in several successful treatment outcomes for liver disorders. However, its use has been severely hampering by many undesirable side effects and methodological restrictions. Currently, there are several advances for the treatment of hepatic diseases with genetic therapy, which address several problems. Research on recent new treatments has focused on the development of specific gene editing approaches that use novel genetic tools, as well as the efficient distribution systems of these tools in the liver. This paper will provide an overview of current and emerging therapeutic strategies such as Adeno-associated Virus Vectors (AAV), new serotypes of AVV for gene therapy and Virus-like particles (VLPs)

Keywords: *Gene therapy, liver diseases, Adeno-associated Virus Vector, Hepatitis E type.*

INTRODUCTION

For many years the scientists have been searching for the solution to several diseases, especially gene diseases related to the liver, but they have only been able to mitigate the symptoms, but not cure them. One treatment development by scientists is gene therapy. Genetic therapy is an experimental technique used for the treatment or prevention of diseases, which consists of introducing genetic material in the cells to compensate for abnormal genes or to make a beneficial protein. The first research about gene therapy has not been 100% reliable. Many clinical essays carried out

in patients reveal that a few percentages of the patient have died. However, in spite of this, the scientist continues to perfect genetic therapy and to show truthful and reliable results for people¹.

One of the major liver diseases related to a virus is hepatitis E type, which is the main cause of hepatitis around the world. This virus caused 44,000 deaths only in 2015. The virus is acquired through under-cooked contaminated meat, especially pork meat. Although several cases of virus acquisition have been reported by air due to direct contact with infected animals². In this paper, the main theme is focused on the implementation of genetic therapies in the treatment of liver diseases such as regeneration of tissues or organs with AAV3 (adeno-associated virus 3). In addition, description de one liver diseases that could be treat where interview vector contrast, vector delivery, and immune response.

Adeno-Associated Virus Vectors and serotypes

The adeno-associated virus was discovered in 1965, being an adenovirus contaminant. It is a virus that does not have a capsid wrapped with a size of 22 nm. The life cycle of AAV is a lytic cycle that carries a line in cells infected with the virus³.

For the production of AAV recombinant two methods are used, the first based on the infection of wild adenoviruses in cell lines that the AAV rep-cap genes and the AAV vector DNA⁴. The second method consists of transient transfection without adenovirus. This technology based on production in milligram quantities of recombinant proteins without the need to create cell lines, consists of using three plasmids: (1) AAV ITR containing plasmid that carries the gene of interest, (2) a plasmid that transports the AAV2 rep-cap, and (3) plasmid that provides the auxiliary genes isolated from adenovirus³.

A brief account of the use of AAV vectors in targeting the liver can explain as a liver-tropical adeno-associated virus vector. As the liver was a considerable subject of treatment with gene therapy. An assay was carried out by Ponnanzhagan, and his colleagues in 1997. Focus on genetic therapy in liver diseases. They could be identified that due to the immune response of the host to the proteins of the AAV2 wrapped capsid to produce a short reaction. The expected results were to show a persistent expression in the study subject, for this trial the hepatic tropism of the rAAV2 vectors was carried out, after the intravenous administration of rAAV2- lacZ vectors in a murine model in vivo⁴.

Serotypes of Adeno-associated virus vector

The genetic therapy based on the adeno-associated virus is an alternative to any liver diseases the research carried out shows that there is hope in the development of gene therapy. This has been evidenced in the first phases of clinical treatments. During the investigations, it was found that the AAV2 vector genome being cross-packaged into variable capsids resulted in new serotypes of AAV virus vector⁵.

In the last years are available several types of AAV serotypes of these could be identified several different types such as AAV10, AAV8, AAV2, AAV3 so that each AAV serotype that performed a different transduction effect in liver cells. Taking into consideration the AAV3 serotype in the selective human liver tropism. A study conducted in non-human primates with AVV3 showed that there was no transduction of any tissue or organ of the study subject⁴.

Serotype	Origin isolates	Receptors ^A
AAV1	Human or NHP	N-linked sialic acid
AAV2	Human	HSPG,FGFR1,HGFR,LamR, and other
AAV3	Human	HSPG,FGFR,HGFR,LamR
AAV4	NHP	O-linked sialic acid
AAV5	Human	N-linked sialic acid,PDGFR
AAV6	Human	N-linked sialic acid,EGFR
AAV7	Rhesus macaque	Unknow
AAV8	Rhesus macaque	LamR
AAV9	Human	LamR, N-linked glycans
AAVrh10	Rhesus macaque	LamR

Data's from Akache et al⁶., Shen et al⁷., Michelfelder and Trepel⁸, showing the origin and receptors for serotype. Abbreviations: AAV, adeno-associated virus; NHP, non-human primates; HSPG, heparin sulfate proteoglycans; LamR, laminin receptor; FGFR1, fibroblast growth factor receptor 1.

Table 1 The origin of common AAV isolates and their receptors

The triad for successful gene therapy

In the last years, the scientists have sought to perfect the method of gene therapy so that it becomes more efficient, for this reason, they have used a “toolbox” for transferring genes. There are three main aspects that could influence the efficacy of gene therapy in hepatocytes, there is expression cassette design (vector construction), the mode of delivery and the immune response¹.



Figure 1. The triad to consider for successful gene therapy

Vector Construction

The selection and design of vector play an essential role in gene therapy for liver diseases. There is three main points in the construction of a correct vector: 1) cDNA transgene, to enhance the transgenic expression, 2) a promoter,

which is important to determinate the level of transgene expression and 3) several post regulatory elements to stabilize trans gene mRNA⁹.

Vector Delivery

The mode of delivery has many challenges to overcome, for instance; although local injection allows a highly selective expression, it only does so when it comes to a small area. On the contrary, an intravenous injection allows a wide balanced distribution. In the case of the liver, the scientists have selected the hepatic artery or the portal vein because these improve the selectivity¹.

On the other hand, the peripheral intravenous delivery gives equal transduction compared with intrahepatic or intraportal routes for AAV (adeno-associated virus). The adeno-associated virus vectors are small viruses, which are been used in gene therapy for their efficiency. These vectors are perfect for gene therapy because it has been tested in several studies with animals and clinical essays¹⁰. This AAV vector will be the main tool to fight against liver diseases

Immune Response

Finally, before of the AAV vector injection into the liver, it is important to evaluate the possible immune responses of the organism to the virus pre-exposition because it could affect the efficacy of gene therapy. In the case of liver gene therapy, the scientist must avoid that the immunologic memory prevents the hepatocytes transduction, for this reason, they apply a recombinant transgenic protein for pre-immunized the transgenic product¹.

VIRUS-LIKE PARTICLES (VLPs) TO HEPATITIS E VIRUS (HEV)

In addition to the use of an adeno-associated virus, there is another kind of virus that can generate the same results in gene therapy for liver diseases, these are the VLPs viruses as a treatment to Hepatitis E virus (HEV). The VLPs have converted into an attractive option due to the ability to mimic the morphology of native viruses. Moreover, VLPs can be used as drug carriers due to their capacity of loading large molecules such as nucleic acids, proteins¹¹.

Scientists have managed to form a VLP through the expression of the terminal N (Nt-ORF2) of the hepatitis virus obtained from mammals. They made a rigorous extraction and purification of HEV-LPs, which at the final, they penetrated the liver cells and tissues. It means that the HEV-LP have the ability to encapsulate the foreign genes and transport to liver-derived cells, and once there, these can be expressed¹¹.

Clinical trials

Clinical trials demonstrate experimentation of the gene therapy used, in this case for the adeno-associated virus vector and the virus as particles have been found several data records where the type of study is specified, the condition or disease, the type of treatment, and the database record where you can find more information. As can be seen in Table 2 most of the studies carried out are in phase 1, that is, the treatments are for their interaction with the human system.

Study title	Condition/Disease	Intervention/treatment	Clinical phase	ClinicalTrials.gov Identifier
Experimental Gene Transfer Procedure to Treat Alpha 1-Antitrypsin (AAT) Deficiency ¹²	Alpha 1-Antitrypsin Deficiency	Biological: rAAV1-CB-hAAT	Phase 1	NCT00430768
HBV Virions Bound Proteins ¹³	Hepatitis B Virus Infection	Other: blood draw	Not Applicable	NCT02798549
HCV Virions Bound Proteins ¹⁴	Hepatitis C	Other: blood draw of 150ml, twice	Not Applicable	NCT02795403
TACE and Adefovir Compared With Transarterial Chemoembolization (TACE) Alone for Hepatitis B Virus (HBV)-Related Unresectable Hepatocellular Carcinoma (HCC) ¹⁵	Hepatitis B Virus Hepatocellular Carcinoma	Drug: adefovir Procedure: TACE	Phase 2	NCT00960518
Safety & Efficacy Study of rAAV1-CB-hAAT for Alpha-1 Antitrypsin Deficiency ¹⁶	Alpha-1 Antitrypsin Deficiency	Drug: rAAV1-CB-hAAT	Phase 2	NCT01054339
Hepatitis C Virus Infection in Patients With Hemoglobinopathies ¹⁷	Hepatitis C, Chronic Hemoglobinopathies	Drug: Antiviral drugs		NCT03149289
Double Filtration Plasmapheresis for Hepatitis C Virus (HCV) Genotype 1 Patients With High Viral Load ¹⁸	Hepatitis C	Drug: DFPP + Peg-IFN + RBV Drug: Peg-IFN + RBV	Phase 4	NCT00977054
A Randomized Controlled Phase 1 Study of Vaccine Therapy for Control or Cure of Chronic Hepatitis B Virus Infection ¹⁹	Chronic Hepatitis b	Biological: Therapeutic hepatitis B vaccine Biological: Commercial Hepatitis B vaccine	Phase 1 Phase 2	NCT03038802
Entecavir Intensification for Persistent HBV Viremia in HIV-HBV Infection ²⁰	HIV Infections Hepatitis B	Drug: Entecavir with the continued standard of care antiretroviral therapy. Drug: continued standard of care with tenofovir in addition to emtricitabine or lamivudine	Phase 4	NCT00662545
Staged Phase I/II Hepatitis C Prophylactic Vaccine ²¹	Hepatitis C	Biological: AdCh3NSmut1, Biological: MVA-NSmut, Other: Placebo	Phase 1 Phase 2	NCT01436357
MRKAd5 HIV-1 Gag Vaccine (V520) in Subjects With Chronic Hepatitis C (V520-022) (COMPLETED) ²²	Hepatitis C	Biological: MRKAd5 HIV-1 gag vaccine (V520), Biological: Comparator: Placebo, Biological: Comparator: Open Label Tetanus and Diphtheria Toxoids Adsorbed	Phase 1 Phase 1	NCT00857311

Table 2. Clinical trials with adeno-associated virus vector and virus-like particles in liver diseases

The genetic treatments are very promising, there are several proposals for treatments for different diseases, especially chronic diseases such as hepatic diseases. This proposal being an efficient alteration in preventive medicine. So, the development and study of new methods such as gene therapy are strategic to improve the quality of life of patients. As we can see in figure 2 you can see the results of the types of treatments: Adeno-associated virus vector and virus-like particles.

GENE THERAPY IN LIVER DISEASE WITH ADENO-ASSOCIATED VIRUS VECTOR AND VIRUS LIKE PARTICLE

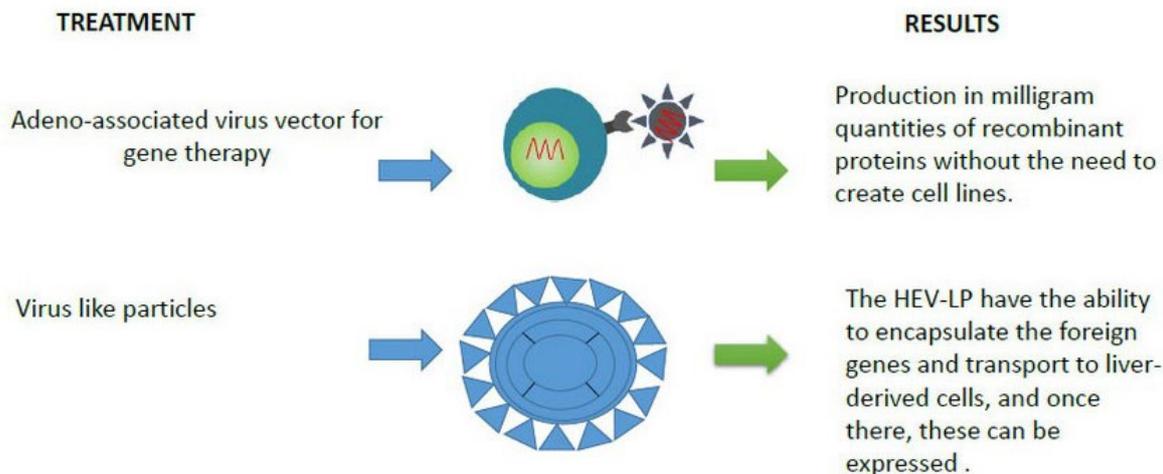


Figure 2. Summarize of gene therapy in liver disease

CONCLUSIONS

Gene therapy studies have made great strides in the last decade, every day the techniques continue to be perfected to cure liver diseases, using the adeno-associated virus and virus-like particles as the main tools. However, there is much to be done especially so that the body adapts perfectly to the newly inserted vectors and does not develop an immune response of rejection towards the same, therefore it is necessary to continue investigating to offer hope of cure patients with liver disease and accessible to everyone. In addition, there are several clinical trials but they are mainly in phase 1 and 2, so more research and clinical trials are necessary since they are scarce.

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