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Innovative Therapeutic Methods for the Treatment of Liver Diseases

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ABSTRACT

The article investigates innovative therapeutic methods for the treatment of liver diseases. The author notes that liver diseases represent a serious burden on health worldwide and they are recognized as the leading cause of mortality and morbidity in the world. In this regard, specialists are constantly searching for innovative methods in the field of treatment and maintenance therapy for liver diseases. Such methods include, for example, mass medical examination of patients at risk, early diagnosis of liver diseases on ultra-precise medical equipment, gene therapy, etc.

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INTRODUCTION

Liver diseases are among the top three in terms of prevalence in the healthcare sector. The average age of death for people with chronic liver disease (CLD) is 50-60 years. In addition, the COVID-19 pandemic has caused the death of patients suffering from these diseases: the risk of death from infection in such patients was 1.5 and was the highest among all other patients suffering from chronic diseases.[1]

Liver diseases are quite effectively detected at late stages, however, currently there is no single diagnostic test sufficient for reliable detection and stratification of early liver disease. Traditionally, a number of blood tests, called «liver functional tests» (LFT), are performed to determine the presence of liver disease. These include enzymes and molecules present in liver damage. These tests are often required, but often do not detect liver disease; up to 20% of LFTs have an abnormal result, but only 1.26% of these patients are later diagnosed with chronic liver disease. Conversely, liver blood tests may be normal in 90% of people with severe liver disease.[2]

Meanwhile, with early detection of the diseases in question, the recovery rate could be quite high. Accordingly, early accurate diagnosis of patients could help to reduce the mortality rate among them and increase the percentage of recoveries.

In this context, the issue of analyzing innovative therapeutic methods of liver treatment at the present stage is quite relevant.

MATERIALS AND METHODS

The analysis of the scientific literature on the subject of the study formed the basis for writing this work. comparative and comparative research methods have also been used.

RESULTS

The complexity of the course of liver diseases and unfavorable outcomes in the anamnesis aroused the interest of specialists in the use of gene therapy (GT).

The gene therapy strategies currently used to treat liver diseases can be divided into 3 main categories. Gene repair involves correcting an existing mutation to restore the expression of the correct version of the protein. Gene addition (or addition) is simply the delivery of the correct version of a gene expressing

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a missing or defective protein. Gene silencing requires the elimination or reduction of protein expression to reduce toxic metabolites.[3]

GT originated in the 1990s as a promising therapeutic alternative to many genetic diseases, including hereditary monogenic diseases. However, GT suffered serious setbacks, including the unfortunate death of a young patient in a clinical trial evaluating the therapeutic efficacy of an adenovirus vector for the treatment of ornithine transcarbamylase deficiency and the development of leukemia in some patients with severe combined immunodeficiency treated with hematopoietic stem cells modified using retroviral vectors. Since then, a period of intensive research has been carried out in this area to develop vectors, new therapeutic methods GT and preclinical safety studies, which eventually led to successful clinical use.[4]

There are two approaches to the organization of gene therapy – *ex vivo* and *in vivo*. In *ex vivo* The patient's GT cells are extracted, cultured, amplified and modified with the selected vector before reinfusion back to the patient. With GT in vivo, the therapeutic agent is administered directly to the patient using various methods of administration, which may be systemic or local. GT in vivo is more applicable for the treatment of liver diseases due to the limited ability to manipulate and multiply hepatocytes *ex vivo*.

Ex vivo gene therapy begins with the isolation of cells that are transduced by a vector carrying a therapeutic gene, and then re-introduced into the body. In vivo gene therapy is based on the direct introduction of a gene delivery vector or genetic material into the body and can use various types of vectors, including non-viral and viral vectors. AAV, an adenoassociated virus.

The liver is a critical organ for most metabolic pathways and, therefore, the source of many hereditary metabolic liver diseases (MLD). A common feature of all these disorders is the lack of synthesis or functionality of proteins involved in biochemical pathways necessary for metabolism.[5]

Traditional enzyme replacement therapy is available only for a limited number of metabolic liver diseases, and the only curative option that confirms the hypothesis of radical treatment is orthotopic liver transplantation (OTP), which is limited by the availability of donor organs and includes lifelong immunosuppression. The fact that OTP may be that restoring the expression of a defective protein in the liver may lead to the resolution of the disease. Thus, the liver-directed strategy of GT and gene editing has become a promising alternative to OTP for metabolic liver diseases.[6]

In addition, substrate reduction therapy (SRT) or metabolic pathway reprogramming are attractive strategies for some metabolic disorders associated with the accumulation of toxic metabolic products. Using small interfering RNA (miRNA) technology or targeted gene editing in order to inhibit key metabolic enzymes, new molecular-based approaches such as genetically determined next-generation SRT or metabolic pathway reprogramming are under development.

It is important to note that the liver is a protein factory that secretes most of the most common proteins present in the bloodstream. Due to this property, the liver is a potential bioreactor for the production of recombinant proteins. This is the case in the case of GT-based treatment of hereditary blood clotting disorders, such as hemophilia A (HemA) or B (HemB), as well as lysosomal accumulation diseases that can be treated with GT.

Due to the large size and negative charge of the genetic material, carriers are needed for delivery inside target cells. There are 2 large families of vectors depending on their origin: viral and non-viral.

Most non-viral vectors consist of polymer or lipid particles that pack and protect the genetic material inside themselves to facilitate entry into the cell. The use of non-viral vectors provides several advantages over the use of viral vectors, such as their easily scalable production, long shelf life, theoretically unlimited payload size of genetic material and a better safety profile. Limitations of non-viral vectors are their low efficiency in penetrating the nucleus and their limited ability to achieve long-term transgene expression.[7]

Each of them has different characteristics, advantages and limitations that are necessary for their selection in a wide variety of gene therapy applications.

The simplest form of introduction of a non-viral vector is the use of naked genetic material. However, this strategy is hindered by the very low efficiency of cell penetration. In the case of hepatocytes, several methods can be used to improve this low transfer efficiency. One of the simplest methods is hydrodynamic injection, based on the rapid introduction of a relatively large volume of solution that causes very high venous pressure in the liver and facilitates the penetration of genetic material into hepatocytes. This method is commonly used to test the concept of research in mice. Although hydrodynamic injections are difficult to transfer to humans, intravascular hydrodynamic procedures with partial catheterization have shown some success in large animals such as pigs, sheep and non-human primates (NHP).

Also, for injection into the cell, other technological methods, such as electroporation or ultrasound, can be performed by DNA. The use of DNA by electroporation in rat liver was first demonstrated in 1996.

Recently, liver electroporation has been used to express α1-antitrypsin (AAT) in mice with AAT deficiency, which has led to a decrease in emphysema. Percutaneous ultrasound has been proven to be effective for delivering genetic material to the liver of mice and pigs. Until now, the use of these approaches in liver-directed GT is still limited by experimental studies on animals.[8]

However, one of the main limitations of the introduction of «naked» DNA, as well as other approaches based on episomal delivery, is the rapid loss of transgene expression due to the loss of genetic material in dividing cells or epigenetic silence. To overcome this limitation, various strategies have been developed, such as the introduction of stabilizing sequences, the use of transposons, and more recently the use of gene editing endonucleases. These strategies have also been used in conjunction with several other delivery methods described below.

Using stabilization sequences is a potential workaround that has been investigated. Scaffold matrix attachment regions

(S/MAR) are genomic DNA sequences that bind chromatin to the nuclear matrix during interphase and are involved in DNA replication and transcription. DNA vectors containing the S/MAR sequence can provide constant mitotic stability in dividing cells and avoid epigenetic silencing, ensuring stable expression of the transgene. However, despite the initial success of this strategy to achieve stable transgene expression in the liver of mice and pigs, S/MAR has not yet been clinically tested.

Two different strategies have been developed for permanent modifications of the cellular genome: transposons and gene editing nucleases. The first are DNA sequences that jump from one place in the genome to another. The transposon sequences are surrounded by terminal inverted repeats (TIR), which are recognized and cleaved by the enzyme transposase, which allows them to be reinserted to other locations. Thus, transposon systems have been modified to integrate therapeutic sequences into the host genome.[9]

Despite the therapeutic interest, the non-targeted integration of transposons should be carefully evaluated due to the potential risk of insertion mutagenesis.

Gene editing tools have changed a lot in recent decades, opening up new possibilities for the treatment of many genetic diseases. The three main platforms are the most promising: zinc finger nucleases (ZFN), effector nucleases similar to transcription activators (TALEN), and clustered short palindromic repeats with regular intervals (CRISPR)/ CRISPR-associated protein 9 (CRISPR/Cas9).

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Some metabolic liver diseases lead to the accumulation of toxic metabolites. One of the most advanced treatment methods is the use of GalNAc-siRNA targeting mRNA aminolevulin synthase 1 (ALAS1) for the treatment of acute hepatic porphyria (AHP). ALAS1 is the first enzyme of hemapute synthesis and its inhibition reduces the accumulation of toxic metabolites and the number of attacks of acute porphyria. The same strategy was used to treat patients with LG1, reducing the level of oxalates in urine by 75%. Products called Givosiran and Lumasiran have recently been approved by the FDA. GalNAc-modified siRNAs are also being developed for the treatment of acquired or hereditary hyperlipidemia, chronic HBV infection, non-alcoholic steatohepatitis (NASH), GSD1a and hereditary hemochromatosis.[10]

The two main classes of non-viral vectors that have shown high efficiency in transferring genetic material to the liver are cationic polymers (polycations) and liposomal compounds.

Cationic polymers form nanoparticles with nucleic acids through electrostatic interactions, ensuring the transport of nucleic acids into the cell. One of the most commonly used is polyethylenimine (PEI), which can provide high efficiency of transduction. Moreover, the use of galactosylated PEI targeting ASGPR proved to be very effective in the transduction of human liver cell lines and the liver of mice and rats. Nanoparticles and derivatives of PEI have been used experimentally to deliver various drugs, including miRNA and microRNA (miRNA), for the treatment of liver malignancies, but clinical studies have not yet been conducted.

Lipid nanoparticles (LNPs) are very similar in composition to cell membranes. They are formed by amphiphilic lipids, which, when dispersed in an aqueous medium, spontaneously form spherical structures with a hydrophilic inner part. LNPs are a suitable carrier for the delivery of nucleic acids due to their excellent biocompatibility, biodegradability, low toxicity and immunogenicity, structural flexibility and ease of large-scale preparation. The use of LNP has significantly revived in GT as carriers for siRNA and mRNA.[11]

Nevertheless, the main problem in the development of LNP-based gene therapy is the search for effective, tissue-specific delivery strategies. Traditionally, targeting is achieved by physically or chemically conjugating ligands for specific receptors on the surface of nanoparticles. The targeting approach was developed by linking a multivalent GalNAc cluster to an LNP. It has been shown that these nanoparticles deliver mRNA molecules to hepatocytes with high efficiency, correcting mouse models of genetic diseases such as methylmalonic acidemia (MMA), PH1, GSD1a, citrine deficiency, acute intermittent porphyria (AIP), etc.

To create a viral vector for GT, the viral genes necessary for replication and those that cause pathogenicity are usually removed from the viral genome and replaced with the genetic sequence that needs to be delivered. Maintaining the replicative ability of the virus is beneficial for some applications, such as oncolytic viruses or viruses that kill tumor cells. Viral vectors that have been used for liver-targeted GT mainly include vectors based on AAV, adenoviruses (Ads), retroviruses (RV) and lentiviruses (LV), since different vectors serve different applications.

DISCUSSION

Taking the interest into account in the topic under consideration, there is an urgent need to focus more efforts on overcoming the problems associated with the implementation of these approaches in the clinic. Among the main problems are the innate and adaptive immune response to the GT product, potential hepatotoxicity and limitations of large-scale and clinical production of vectors.

The introduction of GT vectors does not go unnoticed by the immune system. Both non-viral and viral vectors cause activation of both innate and adaptive immune responses, which can jeopardize the therapeutic effect. In addition, the induction of inflammatory reactions by introducing recombinant vectors into the diseased liver can lead to an exacerbation of liver pathology. In addition, it has been shown that the stable expression of the transgene is limited by the activation of the adaptive immune response, which leads to the elimination of the transduced hepatocytes.[12]

To prevent the disappearance of therapeutic genetic material, a recurring and direct option, already successfully used in both preclinical and clinical trials, is the introduction of immunosuppressants. However, all of them are associated with the risk of temporary weakening of the patient's immunity and, thus, exposure to infection, and depending on the characteristics of the disease, this is a disadvantage that needs to be carefully evaluated. Several alternatives being studied include the use of polymer-coated vectors or modification of the vector surface to prevent APC uptake and reduce immune response activation and increase cellular transduction.

More recently, there has been concern about hepatotoxicity associated with systemic administration of rAAV due to the death of 3 young patients suffering from fatal neuromuscular disorder who received a high dose of vector. Thus, special attention should be paid to the treatment of patients with pre-existing liver diseases, which is typical for patients with metabolic liver diseases.

An additional very important limitation related to immunity is the presence of pre-existing humoral antibodies and Memory T-cells against viral vectors that can completely block liver transduction.

The development of strategies to overcome this problem is the focus of many research groups. Some very simple strategies include using alternative serotypes without cross-reactivity, developing less immunogenic serotypes, or chemical modification of the capsid. Alternatively , physical removal of NAAT by plasmapheresis or immunoadsorption is possible .they have been successfully used on animal models. An interesting NAb elimination strategy based on the use of bacterial proteases capable of cleaving human IgG has recently been reported. This strategy, used to prevent antibody-mediated kidney rejection after transplantation, has been shown to allow repeated dosing of the vector in mice and NHP.

An important aspect that should be carefully evaluated is the oncogenic potential of GT. Accordingly, regardless of the vector used to deliver genetic material, all patients should be closely monitored to monitor possible oncogenic integration. In addition, concrete integration in safe havens may represent a safer strategy that also ensures long-term self-expression.

CONCLUSION

Thus, the liver, due to its central role in metabolism and the role of the protein factory, is a target organ for the treatment of many hereditary and acquired metabolic disorders and represents a very attractive platform for the production of circulating therapeutic proteins. Several approaches to HT aimed at the liver using non-viral and viral vectors provide long-term therapeutic effects. The promising results of ongoing clinical trials aimed at the liver and the authorization to sell a number of GT products inspire optimism about the future of GT in liver diseases.

Author Contributions

All authors contributed in reviewing the final version of this paper.

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