Available online on 15.07.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Review Article

A Review Article on Gene Therapy

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ABSTRACT

Advances in biotechnology have brought gene therapy to the forefront of medical research. Gene therapy can be broadly defined as the transfer of genetic material to cure a disease or at least to improve the clinical status of a patient. One of the basic concepts of gene therapy is to transform viruses into genetic shuttles, which will deliver the gene of interest into the target cells. Safe methods have been devised to do this, using several viral and non-viral vectors. Two main approaches emerged: in vivo modification and ex vivo modification. Retrovirus, adenovirus, adeno-associated virus are suitable for gene therapeutic approaches which are based on permanent expression of the therapeutic gene. Non-viral vectors are far less efficient than viral vectors, but they have advantages due to their low immunogenicity and their large capacity for therapeutic DNA. Gene transfer protocols have been approved for human use in inherited diseases, cancers and acquired disorders.

Keywords: Retrovirus, Lentivirus, Adenovirus, Adeno associated virus.

Article Info: Received 15 May 2019; Review Completed 24 June 2019; Accepted 03 July 2019; Available online 15 July 2019

Cite this article as:

Akki R, Ramya MG, Vardhani, Navyasri K, A Review Article on Gene Therapy, Journal of Drug Delivery and Therapeutics. 2019; 9(4):688-692 http://dx.doi.org/10.22270/jddt.v9i4.3173

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Introduction:

Gene therapy is a novel treatment method which utilizes genes or short oligonucleotide sequences as therapeutic molecules, instead of conventional drug compounds. It is basicallyused to correct defective genes responsible for genetic disorder by one of the following approaches.(1,2)

- A normal gene could be inserted into a nonspecific location within the genome to replace the Nonfunctional gene (most common).
- A n abnormal gene could be repaired through selsective reverse mutation.
- An abnormal gene could be swapped for a normal gene homologus recombination.

Majority of the gene therapy trials are being conducted in united States and europe, with only a modest number in other countries including Australia. Scope of this approach is broad with potential in treatment of diseases caused by single gene recessive disorders (like cystic fibrosis, hemophilia, muscular dystrophy, sickle cell anemia etc), acquired genetic diseases such as cancer and certain viral infections like AIDS.(3,4)

Other gene therapy projects are targeted at conditions such as heart disease, diabetes mellitus, arthritis and Alzheimer's disease, all of which involve genetic susceptibility to illness.(5)

Types of gene therapy:

There are two types of gene therapy

1. Germ line gene therapy:

Where germ cells (sperm or egg) are modified by the introduction of functional genes, which are integrated into their genome. Therefore the therapy would be heritable. Theoretically, this approach should be highly effective in counteracting genetic disease and hereditary disorders. (6)

2. Somatic gene therapy:

Where therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only and will not be inherited by the patients offspring or any later generation. (7)

Approaches of gene therapy:

- Glybera treats one such disease, caused by a defect in lipoprotein lipase.
- DNA must be administered, reach the damaged cells, enter the cell and either express or disrupt a protein.
- Multiple delivery techniques have been explored. The initial approach incorporated DNA into an engineered virus to deliver the DNA into a chromosome. (8,9)

Targeting sites for gene therapy:

Therapeutic genes have to be delivered to specific target sites for a specific type of disease. This table describes the list of such disease and their target sites for gene therapy. (10)

Disease	Targeted cell
ADA deficiency	Blood
Alpha-1-antitrypsin deficiency	Respiratory epithelium
Cancer	Bone marrow,tumor cells
Cystic fibrosis	Respiratory epithelium
Rheumatoid arthritis	Synovial lining cells
Hemophilia B	Skin fibroblasts
Familial	liver

Vectors for gene therapy :(11)

Vectors used in gene therapy can be classified into two types

1.Viral vectors.

2.Non viral vectors.

Viral vectors	Non viral vectors
Adeno virus	Lipid complex
Retro virus	Liposomes
Adeno- associated virus	Peptide/protein
Lenti virus	Polymers
Vaccinia virus	
Herpes simplex virus	

1. Viral vectors:

One of the most promising vectors currently being used is harmless viruses. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the viral genome and replace them with working human gene(12) . This altered virus can then be used to smuggle genes into cells with great efficiency.

Some of the different types of viruses used as gene therapy vectors:

Adenovirus:

Adenoviral vectors are doublestranded, nonenveloped viral vectors that have been the most widely used for gene deliver(13,14). adenoviral DNA does not integrate into the genome and is not replicated during cell division. This limits their use in basic research, although adenoviral vectors are still used in *in vitro* and also *in vivo* experiments. their primary applications are in gene therapy and vaccination. Since humans commonly come in contact with adenoviruses which cause respiratory, gastrointestinal and eye infections, majority of patients have already developed neutralizing antibodies which can inactivate the virus before it can reach the target cell. It is also usedfor the treatment of chronic pain by using a crodent model. These studies helped to establish a CNS treatment paradigm for the treatment of chronic pain(15).

This vector system has been promoted for treating liver cancer and ovaries and indeed the first gene therapy product to be licensed to treat head and neck cancer is Gendicine, p53 based adenoviral product(16). Adeno virus can also infect a broader variety of cells than retro virus.

Retro virus:

First viruses to be used as vectors in gene therapy experiments were retroviruses(17). It is one of the mainstay of current gene therapy approachs. The recombinant retroviruses such as the Moloney murine leukemia virus have the ability to integrate into the host genome in a stable fashion. They contain a reverse transcriptase to make a DNA copy of the RNA genome, and an integrase that allows integration into the host genome. They have been used in a number of FDA-approved clinical trials such as the SCID-X1 trial(18).

Retroviral vectors can either be replication-competent or replication-defective. Replication-defective vectors are the most common choice in studies because the viruses have had the coding regions for the genes necessary for additional rounds of virion replication and packaging replaced with other genes, or deleted.These virus are capable of infecting their target cells and delivering their viral payload, but then fail to continue the typical lytic pathway that leads to cell lysis and death.

Replication-competent viral vectors contain all necessary genes for virion synthesis, and continue to propagate themselves once infection occurs. Because the viral genome for these vectors is much lengthier, the length of the actual inserted gene of interest is limited compared to the possible length of the insert for replication-defective vectors. Depending on the viral vector, the typical maximum length of an allowable DNA insert in a replication-defective viral vector is usually about 8–10 kB(19).

There is concern that insertional mutagenesis due to integration into the host genome might lead to cancer or leukemia. This concern remained theoretical until gene therapy for ten SCID-X1 patients using Maloney murine leukemia virus(20) resulted in two cases of leukemia caused by activation of the LMO2 oncogene due to nearby integration of the vector(21).

Adeno associated virus:

One of the most promising potential vectors is a recently discovered virus called the AAV, which infects a broad range of cells including both dividing and non dividing cells. AAVs are small viruses from the Parvovirus family with a genome of single stranded DNA. It can insert genetic material at a specific site on chromosome 19 with near 100% certainty.Researchers believe that most people carry AAV which do not cause disease and do not provoke an immune response.Scientists have demonstrated the animal experiments using AAV to correct genetic defects(22).

Also clinical trials have been initiated to use AAV vectors to deliver genes to brain as the virus can infect nondividing cells like neurons in which their genome are expressed for a long time.The chief drawback of AAV is that it is small, carrying only 2 genes in its natural state. Its payload therefore is relatively limited. It can produce unintended

genetic damage because the virus inserts its genes directly into host cell's DNA. Researchers have also had difficulties in manufacturing large quantities of the altered virus. The production problem has recently being solved by Amsterdam Molecular Therapeutics(23).

Lenti virus:

Lentivirus are a subclass of Retroviruses. They are sometimes used as vectors for gene therapy they having a great ability to integrate into the genome of non-dividing cells, which is the unique feature of Lentiviruses as other Retroviruses can infect only dividing cells. The viral genome in the form of RNA is reverse-transcribed when the virus enters the cell to produce DNA, which is then inserted into the genome at a random position (recent findings actually suggest that the insertion of viral DNA is not random but directed to specific active genes and related to genome organization)(24) by the viral integrase enzyme. The vector, now called a provirus, remains in the genome and is passed on to the progeny of the cell when it divides.

However, studies have shown that lentivirus vectors have a lower tendency to integrate in places that potentially cause cancer than gamma-retroviral vectors(25).

More specifically, one study found that lentiviral vectors did not cause either an increase in tumor incidence or an earlier onset of tumors in a mouse strain with a much higher incidence of tumors(26).

Vaccinia virus:

Vaccinia virus (**VACV** or **VV**) is a large, complex, enveloped virus belonging to the poxvirus family(27).It has a linear, double-stranded DNA genome approximately 190 kbp in length, and which encodes approximately 250 genes. The dimensions of the virion are roughly 360 × 270 × 250 nm, with a mass of approximately 5-10 fg(28).

Vaccinia virus is closely related to the virus that causes cowpox; historically the two were often considered to be one and the same(29)oxviruses are unique among DNA viruses because they replicate only in the cytoplasm of the host cell, outside of the nucleus. (30)

Therefore, the large genome is required for encoding various enzymes and proteins involved in viral DNA replication and gene transcription. During its replication cycle, VV produces four infectious forms which differ in their outer membranes: intracellular mature virion (IMV), the intracellular enveloped virion (IEV), the cell-associated enveloped virion (CEV) and the extracellular enveloped virion (EEV)(31).

Herpes simplex virus:

Herpes simplex virus 1 and **2** (**HSV-1** and **HSV-2**), also known as **human herpesvirus 1** and **2** (**HHV-1** and **HHV-2**), are two members of the human *Herpesviridae* family, a set of viruses that produce viral infections in the majority of humans(32,33). Both HSV-1 (which produces most cold sores) and HSV-2 (which produces most genital herpes) are very common and contagious. They can be spread when an infected person begins shedding the virus. About 67% of the world population under the age of 50 has HSV-1(34). In the United States more than one-in-six people have HSV-2(35). Although it can be transmitted through any intimate contact, it is one of the most common sexually transmitted infections.(36)

Non-viral vectors:

Non-viral methods present certain advantages over viral methods, with simple large scale production and low host immunogenicity being just two. Previously, low levels of transfection and expression of the gene held non-viral methods at a disadvantage; however, recent advances in vector technology have yielded molecules and techniques with transfection efficiencies similar to those of viruses.(37)

Injection of naked DNA:

This is the simplest method of non-viral transfection. Clinical trials carried out of intramuscular injection of a naked DNA plasmid have occurred with some success; however, the expression has been very low in comparison to other methods of transfection. In addition to trials with plasmids, there have been trials with naked PCR product, which have had similar or greater success. Cellular uptake of naked DNA is generally inefficient. Research efforts focusing on improving the efficiency of naked DNA uptake have yielded several novel methods, such as electroporation, sonoporation, and the use of a "gene gun", which shoots DNA coated gold particles into the cell using high pressure gas.(38)

Electroporation:

Electroporation is temporary destabilization of the cell membrane targeted tissue by insertion of a pair of electrodes into it so that DNA molecules in the surrounding media of the destabilized membrane would be able to penetrate into cytoplasm and nucleoplasm of the cell(39.40)but unfortunately the trangene can integrate only to 0.01% of the treated cells.(41) Electroporation has been used *in vivo* for many types of tissues, such as skin, muscle, lung(42-44) There are some problems in this method too that the more important are the difficulty in surgical procedure in the placement of electrodes into the internal tissues and that the high voltage applied to tissue might damage the organ and affect genomic DNA stability.(45)

Hydrodynamic:

Hydrodynamic is a simple and highly efficient method for direct intracellular delivery of any water-soluble compounds and particles into internal organs(46) The efficiency of this simple method *in vivo* is higher than any other nonviral system. This method has been successful for gene delivery into rodent liver and expression of hemophilia factors, (47)cytokines,(48)erythropoietin,(49)and hepatic growth factors,(50) in mouse and rat but it has been successful only in small animals and not in human.

Ultrasound:

Ultrasound can make some nanomeric pores in membrane to facilitate intracellular delivery of DNA particles into cells of internal organs or tumors, so the size and concentration of plasmid DNA have great role in efficiency of the system. (51,52) The most important limitation of the system is low efficiency of it, especially in vivo.

Advantages of gene therapy:

- In case of 'silence' a gene. In the case of someone with HIV, which had not yet developed into AIDS, scientists could save them the pain and suffering of the disease by using gene therapy to 'silence' the disease before its onset.

- Gene therapy has the potential to eliminate and prevent hereditary diseases such as cystic fibrosis and is a possible cure for heart disease, AIDS and cancer.
- These sceptics would almost certainly choose gene therapy, especially if it was the last hope for them or one of their loved ones – as is the case for many gene therapy patients. (53)

Disadvantages of Gene Therapy:

- Short-lived nature of gene therapy.
- Immune response - Genes injected with a virus may trigger an immune response against the virus.Problems with viral vectors (once inside the patient, the viral vector could recover its ability to cause disease).
- Multigene disorders - The genetic material might not get into the right cell, or the right place in the cell's DNA. (54)

Conclusion:

ene therapy is the one of the exiting and ultimate application of DNA sciences. This therapy will be applied, depends on the simplification of procedure. By using gene therapy many diseases are cured in present days like cancer, AIDS, hepatitis, melanoma, alizhmers, and parkinsons. In future gene therapy is the treatment for many diseases.

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