Review Article

DNA VACCINE: A MODERN BIOTECHNOLOGICAL APPROACH TOWARDS HUMAN WELFARE AND CLINICAL TRIALS.

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Abstract
Vaccination is need of today’s world for prevention of diseases. Vaccines reduce the mortality rates in the world from infectious diseases such as measles, polio and diphtheria. The concept of vaccination is very old. Conventional vaccines (First generation vaccines) are composed of Live or Attenuated microorganisms. But they may have some problems, so further research is going on for development of a vaccine which cost-effective and having specific immune responses. DNA vaccines are third generation vaccines and protect an organism from diseases by injecting it with genetically engineered DNA. DNA vaccines are made up of bacterial plasmid. DNA vaccine is able to produce both humoral and cell-mediated immunity. A single DNA vaccine is able to produce immunity for two or more diseases. In this review we are discussing about the preparation, insertion, mechanism, advantages and disadvantages of DNA vaccines. DNA vaccines have a bright future ahead.

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KEYWORDS: DNA Vaccine, Gene gun method, Mechanism of Action, Future.

INTRODUCTION
WHO estimated that 80% of illness in the world is due to various diseases which cause more than 20 million deaths per year [1]. Vaccines play a major role in prevention of diseases. Vaccination is a cost-effective measure for disease prevention. Vaccines reduce the mortality rates in the world from infectious diseases such as measles, polio and diphtheria.

The concept of vaccination has been around for centuries. Edward Jenner and Louis Pasteur were made first attempt for human diseases. A powder was derived from crusts of small pox lesions and used first time in 15th century. This powder was inserted into body with the help of a pin or “poking” device [2]. This process was called as variolation. These practices were not meant to save human lives but used for preserve the beauty of a young woman. After that vaccination was originated, when Edward Jenner created the first successful vaccine against small pox in 1796. He injected the infectious material from a woman with cowpox into the arm of young boy; the boy became resistant from life-threatening viruses [3]. Smallpox was the first disease which has been prevented by scientists by intentionally inoculating individuals at risk with infecting agent [4]. In 1885, Louis Pasteur became involved in the practices of immunization and has been worked on the vaccines for Anthrax and Rabies. Pasteur created an attenuated form of virus and used for immunization [5, 6]. Vaccines are composed of Antigens that artificially induces the body’s immune system to produce antibodies, so that body become resistant against particular disease.

Conventional vaccines (First generation vaccines) are composed of Live or Attenuated microorganisms. They requires whole microorganism for vaccine preparation. Although vaccines are very useful for disease prevention, but the conventional vaccines also have some problems such as the attenuated forms of a pathogen can revert to a harmful form and may still be able to cause disease. So, to reduce the risk rates second generation vaccines were developed. These are subunit vaccines, composed of defined protein antigens or recombinant protein components [7].

DNA vaccines are called as third generation vaccines. Recombinant DNA technology plays an important role in preparation of these vaccines. DNA vaccines are made up of small, circular piece of bacterial DNA that has been genetically engineered for the production of two or more antigens from a single pathogen. When vaccine DNA is inserted into the host cells of body, the “inner-machinery”
of cells converts that DNA into the pathogenic proteins. These proteins are recognized as foreign, then the immune system triggers a range of immune responses [8, 9].

**MOLECULAR TOOLS FOR DNA VACCINES**

DNA vaccines are prepared on the basis of Recombinant DNA technology. RDT is used for preparation of gene sequences which are not found in biological organisms. These sequences are prepared by transferring genetic materials (DNA sequences) one organism to another organism. In this technology basically following tools are using:-

**Plasmid (Vectors):-** Plasmid is a DNA molecule, which has capacity of replication independently of the chromosomal DNA [22]. Plasmids are double stranded in nature and usually are circular. Plasmids occur naturally in bacteria, but sometimes are present in eukaryotic organisms. Plasmid is an important tool for preparation of DNA vaccines. Plasmids are generally using for multiplication of gene of interest by inserting the desired gene into the plasmid.

**Nucleases:** - Nucleases degrade DNA molecules by breaking the phosphodiester bonds that binds nucleotides to each-other in a DNA strand. These are of two types: Endonucleases and Exonucleases. Exonuclease removes one nucleotide at a time from the end of a DNA molecule. While Endonucleases are able to break internal phosphodiester bonds within a DNA molecule.

**Ligases:** - DNA ligase enzyme uses for repairing of single stranded breaks in double-stranded DNA and also joins together two individual fragments of double-stranded DNA.

**DNA modifying enzymes:** - Several enzymes modify DNA molecules by addition or removal of specific chemical groups. Some are as following:-

- **Alkaline Phosphatase** - It removes the phosphate group present at 5’ terminus of a DNA molecule.
- **Polynucleotide kinase** - It has the opposite effect to alkaline phosphatase.
- **Terminal deoxynucleotidyl transferase** - It adds one or more deoxynucleotides onto the 3’ terminus of a DNA molecule.

**Topoisomerasers:** - The final tool for recombinant DNA technology is topoisomerase. These enzymes are able to change the conformation of covalently bind-circular DNA (plasmid) by introducing or removing supercoiling.

**PREPARATION OF DNA VACCINES**

DNA vaccines are made up of bacterial plasmid [Fig 1]. Plasmid which is used in DNA-based vaccines normally has two units: Antigen expression unit and The Production unit. Antigen expression unit is made up of promoter sequences, followed by antigen-coding and polyadenylation sequences. The Production unit is composed of bacterial sequences which are useful for amplification and selection of plasmid [10]. Once the vaccine plasmid is constructed, then it is transferred into bacteria. Then this DNA acts as the vaccines [11].

**INSERTION OF DNA VACCINE INTO ANIMALS**

Vaccinated DNA can be transferred into animal tissues by several methods. Two most useful methods are injection of DNA in saline, by using a hypodermic needle and Gene gun method. DNA in saline is normally injected intramuscularly (i.m.) in skeletal muscle, or intradermally (i.d.) to the extracellular spaces. This procedure can be done by Electroporation [12], by temporarily damaging muscle fibers with myotoxins like bupivacaine; or by using saline or sucrose solutions [8]. Immune response evoked by DNA vaccine can be affected by several factors, such as needle type [13], needle alignment, speed of injection, volume of injection, muscle type, age, sex and physiological conditions of animal being injected [8]. Gene gun method is the most common method of plasmid DNA insertion. In this method plasmid DNA is absorbed on to Gold or tungsten micro particles into the target cells [8,14].This process is also calledas Micro projectile [Fig 2]. Various amounts of DNA can be transferred according to the method of insertion. By saline injection 10µg-1mg and by gene gun method 100 to 1000 times less then intramuscular saline injection can be injected [15].

**MECHANISM OF ACTION OF DNA VACCINES**

When a plasmid DNA is injected into skin or muscle, then protein is produced endogenously and intracellular as small antigenic peptides by the proteases of the host. These peptides then transferred to the lumen of the endoplasmic reticulum (E.R.) by membrane associated transporters. In the E.R., these peptides bind with class-I MHC molecules. Then these peptides are presented on the cell surface of class-I MHC molecules and stimulate CD8+ cytotoxic T-cells (CTL) and they evoke cell mediated immunity. CTL inhibit viruses through two processes- cytolysis of infected cells and non-cytolysis like cytokinin production [16].
The antigenic proteins can also be presented by class-II MHC molecules. In this process antigen presenting cells stimulates the CD4+ helper T-cells. These CD4+ cells can recognize the peptides of exogenous proteins that were endocytosed by APCs, then these peptides degraded into fragments and loaded onto class-II MHC molecules. By the CD4+ cells, B-cells are stimulated and enhance the antibody production [10].

Fig.3. MECHANISM OF ACTION OF DNA VACCINE [23].

ADVANTAGES OF DNA VACCINES
DNA vectors play an important role in clinical applications, where large-scale production is not easily managed by conventional vaccines and other vaccines [17].

Viral mediated gene transfer by genetically modified lentiviruses, adenoviruses and retroviruses is very useful because it has high transfection efficiency and stability [18].

DNA vaccines have no risks like conventional vaccines. Immune response can be evoked by both class-I and class-II molecules. This is a unique feature of DNA vaccines [19].

It is possible to make a single DNA vaccine which can encode for several antigens or proteins.

DNA vaccines are safe than live attenuated which can cause infection in vivo. It is studied that after multiple immunization anti-DNA antibodies are not produced [20].

Oligonucleotides can also be transferred by DNA vaccines. These oligonucleotides can alter gene splicing or gene expression such as siRNA [21].

LIMITATIONS OF DNA VACCINES
Major limitation of DNA vaccines is that these are useful only for protein antigens. DNA vaccines cannot be used for non-protein based antigens.

A major risk with DNA vaccine is that they can affect the genes which control the growth of cells.

It may be possible to inducing antibody production against DNA.

Tolerance can be generated to the antigens produced by DNA vaccines.

FUTURE OF DNA VACCINES AND CONCLUSIONS
The field of DNA vaccines is big and it will continue to advance and new approaches to enhance the immunogenicity of DNA vaccines. DNA vaccines could help in improving applications for gene therapy and gene vaccination. Improved gene expression and better genetic engineering of plasmid DNA may increase antibody response to the gene products.

The DNA vaccination is a new branch of medical sciences. Studies and research are going on but some DNA vaccines have moved to clinical trials. In this review we have discussed that how DNA vaccines are advanced then conventional and second generation vaccines. Antigen expression of DNA vaccines can be improved by inclusion of Adjuvant in the formulation, or as immune modulators to improve the immunogenicity. If immunotherapy is combined with chemotherapy and radiotherapy, then it could yield systemic or additive therapeutic results.

In future, DNA vaccines may prepare by noval approaches in the form of microspheres, nanobeads or micro-nanoporation. So vaccination will become painless, effective and safe needle-free routes such as the intranasal or the oral route. These nanovaccines are in experimental stage at present but may have a great future ahead.

DNA vaccines are able to produce more than one antigen, so we can make one medicine for many diseases. DNA vaccines against lethal diseases such as AIDS, Cancer, Rabies, Malaria and Diabetes can be available. Now-a-days DNA vaccines are in their early phase but one day is going to be the vaccines of next generation.

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