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'Pharming' proteins: the future of Panacea

Deepali Gangrade*, Nimisha Waghmare and Sayali Lad

Department of Pharmaceutical Chemistry, Vivekanand Education Society's College of Pharmacy, Chembur (E), Mumbai- 400074, (M S)India

ABSTRACT

As an attempt to remove the obstacle of sickness and disease, human created medicines to cure ailments. As this wasn't enough the term Genetic Modification came into existence. With the advances in genetic engineering, it has now become possible to produce proteins outside the body with the help of molecular pharming techniques. This has opened the gates for producing wide range of medicines from antibody to antigens. Immunity related problems have always been a major concern in our society. Molecular pharming has largely revolutionized the production of immunotherapeutics in today's market.

Keywords: Genetic modification, genetic engineering, proteins, molecular pharming, antibody, antigen, immunity, immunotherapeutic

INTRODUCTION

Recently, there has been a vigorous development in the use of vaccines, antibiotics, analgesics, hormones and other pharmaceuticals. The advent in the biotechnological era has led to the development of recombinant molecules. The basic need for the production of such medications is the proteins. Proteins are said to be the building blocks of the body as well as the building blocks of the upcoming medications. These are the most important necessity of pharmaceutical industry [1]. Proteins control most of the mechanisms in the body in the form of enzymes, hormones, antibodies, antigens, etc. As a result, these proteins are required in abundance when the body functions are abruptly interfered due to changes in the levels of proteins. While short peptide chains are produced chemically, larger proteins are best produced by living cells. Genetic engineering provides the best suitable means of producing proteins through recombinant technology. One of the most widely used protein manufacturing technique is "Molecular Pharming" [2].

Molecular pharming:
It is the process of producing useful pharmaceuticals by inserting genes into plants or animals thus creating genetically modified organisms [GMOs]. Molecular pharming is basically used for the production of recombinant proteins [2]. Generally plants are used to produce GMOs as they are low in cost and efficient. Animals and microbial cell cultures are also used to produce recombinant proteins but the cost associated with maintenance and safety is higher.

General pharming strategy:
The process of molecular pharming involves various steps:

- Initially, the genetic information needed to be produced is carried on to the DNA molecule.
- It is then inserted into the host species where it becomes a part of its genome [3]. The host species which is used to carry out the multiplication process is called as the expression system. The expression system should be selected efficiently. It must be safe, efficient, cost effective and harmless and should produce the biomass within short

duration [4]. Various host systems are used for this purpose including recombinant micro-organisms, transgenic plants and animals [5].

- The protein which is expressed onto the DNA is recognized and further multiplied by the protein-making machinery.
- In this way, the recombinant proteins are produced, which are further extracted and converted into the pharmaceutical product [3].

Pharming with the greens:

Plants are the major bio-factories in the production of recombinant proteins [6].

Advantages of using plants over other host systems [7]:

- It involves less cost as the bioreactors and other costly equipments are not required.
- The product is obtained in abundance as the plants can be cultivated in large acres of land.
- They have better stability during storage for longer periods and cold storage facilities are not required.
- Cells in the fermentation tank can catch human diseases, whereas plants do not.

Building immunity with plants:

Today's population is largely suffering on the cost of immunity. Immune system disorders are generated due to over activity of immune system i.e. the body attacks and damages its own cells or low activity i.e. body's capability of combatting foreign invaders decreases resulting in increased vulnerability to infectious diseases [8].

The concept of using plants as an expression system for developing immunotherapies led to a major advancement in the field of medical sciences. Plant systems are widely used for producing antigens, antibodies, and cytokines in large amounts.

A. Plantibodies:

The combination of antibodies and plant engineering, two rapidly advancing technologies, has led to emerging strategies in diversified plant species [9]. Antibodies are the immunoglobulins that are used by the immune system to identify and destroy the pathogenic viruses and bacteria. They are classified as IgG, IgM, IgA, IgD and IgE. They are specifically released by the B-cells of immune system [10]. The antibodies produced in plants are termed as "Plantibodies". Plantibodies are the plant products that are expressed genetically to produce antibody and antibody fragments [11].

The production of plantibodies involves two methods:

i.Biolistics

ii.Agrobacterium mediated transformation

i.Biolistics:

In biolistic, the genes encoding for specific antibodies are impregnated into nucleic acid or other biological molecules by coating them with certain carrier systems called as the micro-carriers.

These micro-carriers could be:

- High density gold microparticles
- High density tungsten microparticles

A gene gun is used where these coated particles with the help of helium pulse are accelerated with a high veloctity. These particles are bombarded which then are able to penetrate into the cell walls and the membranes and thus enter into the cell [12].

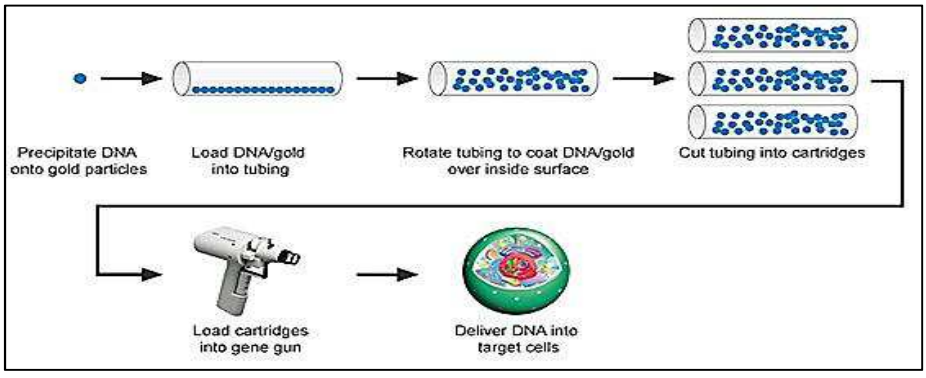

Figure 1: Biolistic method for plantibody production [13]

ii.Agrobacterium mediated transformation:

The gene of interest is injected into the Ti-plasmid of *Agrobacterium Tumefaciens*. This bacteria is then allowed to infect the plant. This results in the formation of tumours within the plant called as the Crown Galls disease of the plants. The bacteria transfers a tumour-inducing (Ti) plasmid located in a section of its DNA (known as T-DNA) into the nucleus of an infected plant cell [14]. The newly introduced Ti-plasmid is incorporated into the plant genome and is consequently transcribed. The T-DNA that is integrated into the plant genome contains cancer-causing oncogenic genes and genes that produce opines which are excreted by infected Crown Gall cells and are a food source for *Agrobacterium tumefaciens.* Oncogenes and opine creating genes can be removed from the Ti-Plasmid that is transferred to the plant cell by T-DNA. From the plant cell cultures the plantlet is grown from which the proteins are extracted and purified [15].

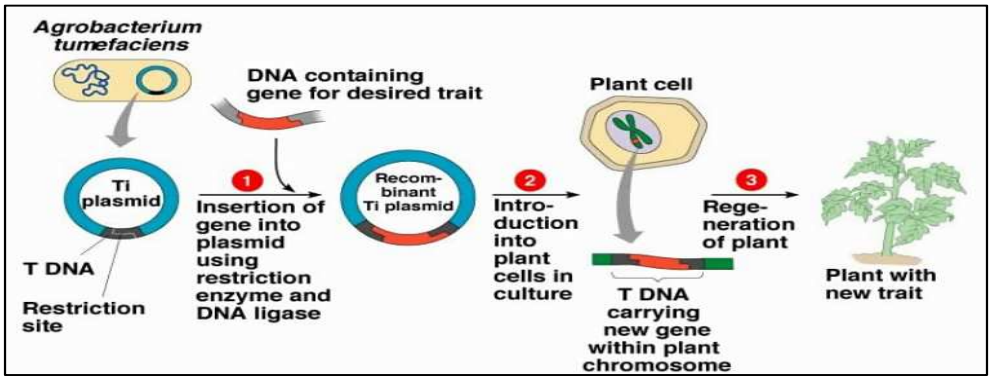

Figure 2[16] Agrobacterium mediated transformation

Plantibodies for cancer therapy:

Plants have been chosen to make monoclonal antibodies for cancer therapy as excess can be produced. Genetically engineered soybean has been made which produces monoclonal antibody (BR-96), thus acting as a vehicle for targeting doxorubicin for ovarian, breast, lung and colon tumours. Monoclonal antibody for Non-Hodgkin's lymphoma is also under investigation [9].

Case study:

Fight against the Ebola virus:

The 2014 Ebola epidemics are the major in history, hitting large number of countries in West Africa [17]. Ebola is a deadly disease resulting from the attack by the Ebola Virus. The symptoms include fever, sore throat, muscle pain, and headaches [18].

Till now Ebola virus was combatted with the help of monoclonal antibodies in recombinant mouse cells. The mice is infected with a protein from the Ebola virus, and then the drug is produced by modifying the mice's antibodies to more closely resemble human ones [19]. The drug, ZMapp then was developed which is actually the alliance between an American company, Mapp Biopharmaceutical, and a Canadian company funded by the Public Health Agency of Canada. ZMapp is made up of proteins referred as monoclonal antibodies, which bind to the Ebola virus rendering it harmless [20].

Drawback:

- Human immune system identified these antibodies as a foreign particle and hence was not effective.
- Plants were preferred over animals because they are free of mammalian virus vectors.

Also, the need for these antibodies was high. Hence, the scientists came up with the new idea of producing these antibodies in plants [21]. A gene from the modified antibodies was introduced into the leaves of tobacco plants, via a system developed by Icon Genetics. The leaves then produced the intended monoclonal "plantibody" proteins. It only took about a week before the leaves could be harvested and the protein extracted and purified. Plus, it was found to be economical as compared to the traditional method of growing genetically modified animal cells in labs.
Currently these antibodies are being grown in tobacco plant at Kentucky Bioprocessing, a unit of tobacco giant Reynold American [20].

ZMapp:

It is a mixture of three monoclonal antibodies c13C6, c2G4 and c4G7.
1.Gene encoding for the chimeric mAbs is inserted into host bacterium.

2.This bacterium is then allowed to infect the plant Nicotiana Benthanmianas.
3.The target protein is produced which is then harvested and processed.
4.This is further purified to give the pharmaceutical product [22].

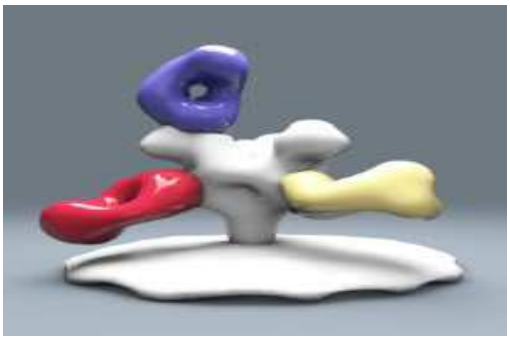

Figure 3: ZMapp drug model [23]

Mechanism of action [24]:
1.The filamentous membrane of the Ebola virus has many glycoproteins embedded within it.
2.These glycoproteins are essential for the binding of the ZMapp.
3.The ZMapp recognizes the glycoproteins on Ebola virus.
4.It gets attached to these virus cells.
5.This results in the activation of an immune response against these tagged viruses.
6.Further, the virus-Ab complex attracts other immune cells, which attacks the virus.

Approval process:
The drug so far has only been produced in small quantities since as it has not undergone clinical trials in humans but attempts are made whether it should be made more widely available to people stricken with the virus in West Africa. The ZMapp was used in patients affected with the Ebola virus in America [25]. It was an emergency condition; hence the drug was given without performing clinical trials. It was found to be effective in 2 patients. Also, monkeys infected with the virus showed 100% effectiveness towards ZMapp [26]. So measures have been taken to produce the drug in large quantities.

A.PLANTIGENS:
Plantigens are antigens that are produced in plants [27]. These are used for the preparation of vaccines. Vaccines have been proved to be miraculous in treating various infections [28]. Conventional vaccines include attenuated or inactivated pathogens [29]. Gene which encode for a particular antigenic peptide is isolated and then expressed in bacterium which then infects the plants thus producing vaccines. Plant vaccines are comprised of antigenic proteins and do not contain pathogenic genes. Gene which encode for antigenic peptide is placed under a constitutive promoter. Appropriate plant parts containing antigen can be administered to humans to bring about immunization [30].

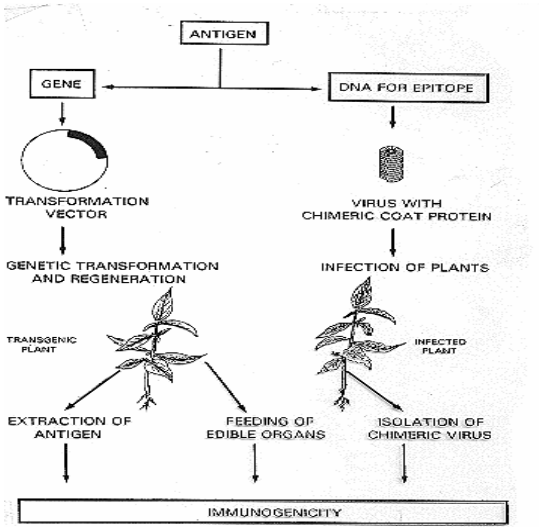

Figure 4: Flowchart depicting production of plantigen [31]

Edible vaccines:
Scientists thought of the alternatives that vaccines could be produced in edible parts of the plant rather than the inoculations. These vaccines could then be directly consumed to bring about the immunization in the body. Food vaccines are such that they contain antigens but do not contain the whole pathogens [28].

The approval process:
The experiments have demonstrated that tomato or potato plants have shown to produce the antigens for Vibrio cholerae Norwalk virus, Escherichia coli and Hepatitis B virus. Animals on feeding with these edible vaccines evoked mucosal and systemic immune responses that provided protection from exposure to the real pathogens [28]. The biggest question is that whether such edible vaccines would be able to survive in the gastro-intestinal tract of human beings.

Some edible vaccines that are making its way to the market:
a.Transgenic potatoes for diarrhoea:
The first human trials with this edible vaccine were carried out in 1997. Transgenic vaccine containing b-subunit of E. coli heat-labile toxin was fed to the volunteers that caused diarrhoea. Out of 11, 10 volunteers showed an increase in the production of antibodies. However, the disadvantage is that the potato cannot be eaten raw. Hence, cooking denatured some proteins in the vaccines but on partial boiling, half the amount of proteins was still alive [32].

b.Transgenic tomatoes against diarrhoea:
Researchers have developed transgenic tomatoes using Norwalk virus which causes diarrhoea. The tomatoes were shown to produce surface proteins specific to the virus.
Banana is the best alternative for producing edible vaccines because not only does it eliminate the need for cooking but also it is grown locally all over [32].

Advantages [33]:
- Effective as a delivery system for bringing out the immunization process
- Better patient compliance
- Can be self-administered
- Involves low cost
- Antigen protection through bio-encapsulation
- Ease of mass production

Disadvantages [32]:
- The problems related to the dosage control
- The survival of vaccines in the acidic and gastric environment of stomach
- Glycosylation pattern in plants differ from humans. Hence, they could affect the functionality in the body.
- Development of allergic responses

CONCLUSION

Molecular pharming has revolutionized the pharma industries. Increased pharmaceutical demands, as well as advances in gene identification have led to an interest in plants as expression systems for therapeutic products. The quest for newer drugs is never ending as is the need to understand disease causes beyond the symptoms. Molecular pharming provides a huge scope for economical and large scale production of pharmaceuticals, industrial enzymes and technical proteins that are currently produced at higher cost and in limited quantities. Also, building immunity is now possible with molecular pharming. Such a large scale production of immunotherapeutic has now raised hopes in the minds of underprivileged. With this increased productivity and potentially lower economy, more patients will be able to receive the drugs they need. Despite numerous advantages to this approach, however, the concerns that have been raised must be adequately addressed. We must make sure that these benefits are not outweighed by risks to human health and the environment. Although molecular pharming offers an exciting alternative for pharmaceutical production, industry and government must proceed cautiously in this area in order to gain public acceptance.

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