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Review Article

Edible Vaccines: A new Approach for Immunization in Plant Biotechnology

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Abstract: Vaccines are most widely used in the world. They have reduced the mortality rate caused by various infectious organisms. Prevention of diseases is the most appropriate approach to health. Vaccines trigger and prepare our body's defense mechanisms so that the system is able to fight and eliminate the pathogens when encountered due to natural infection. But a mind disturbing reality unrecognized is the ever growing no of individuals suffering from adverse reactions to vaccines. Each year, millions of children in underdeveloped countries have no access to immunization. The traditional vaccines are expensive and, require special conditions for storage, distribution and & dispensing. Edible vaccines can be one of the alternatives of the traditional vaccines. Edible vaccines are cheaper, easy-to-administer and store, readily acceptable socially; especially for the poor and developing countries. The technique for production of edible vaccines involves introduction of selected desired genes of immunogenic proteins from various pathogens into plants and animals and then inducing these altered organisms to manufacture the encoded proteins. Edible vaccines are targeted to provide mucosal as well as systemic immunity. The future of edible vaccines depends on acceptability for genetically modified foods. Successful implementation of edible vaccines relies on how well we overcome various technical obstacles, regulatory issues and non-scientific challenges. This paper reviews the method of preparation, mode of action, advantages, limitations, applications, clinical trails related to edible vaccines which can create significant impact on promotion of global health.
Keywords: Edible vaccines; global health; immunogenic proteins; transgenic organisms

Introduction

We all are prone to one or more kind of infections throughout our life. To prevent this infection we discovered vaccine, route of administration into our body is injection in most of the cases. But in the future this will change; having a Banana will give immunity against diarrhoea caused by E.Coli. As Hippocrates said, "Let thy food be thy medicine," scientists suggest that plants and plant viruses can be genetically engineered to produce vaccines against diseases [1]. Vaccines are biological preparations introduced into healthy individuals that aim at triggering and training of their immune system to fight against disease causing agents when encountered naturally. It is well known that extensive use of small pox vaccine has helped to eradicate the disease. A massive polio eradication program has been successful in many countries and ongoing in India. The various vaccines can be grouped as *Conventional* involving introduction of killed or live but inactivated forms of pathogens produced by chemical or physical treatment like BCG and, oral polio vaccine etc. They are easy to produce at low cost but can revert to infective forms and cause disease if not stored and administered properly; *Purified antigen vaccines* aimed at introduction of polysaccharide or protein purified from causative agents as toxoids of tetanus, diphtheria, gangrene etc and *Recombinant vaccines* produced by recombinant DNA technology and include Subunit vaccines, DNA vaccines and Edible vaccines. They are produced by identification and isolation of genes encoding immunogenic proteins of pathogenic organism followed by cloning and expression of the gene in suitable host organism for mass production of the concerned protein.

Types of Vaccines

Vaccines may be dead or inactivated organism s or purified products derived from them.
There are four types of traditional vaccines.
Vaccines containing killed micro organisms
Eg: vaccines against flu, cholera, bubonic plague and he patitis A.
Vaccines containing live, attenuated virus micro organis ms.
Eg: Yellow fever, measles, rubella and mumps
Toxoids – These are inactivated toxic compounds
Eg: Tetanus and diphtheria
Protein subunit -
Rather than introducing an inactivated or attenuated mi cro organisms to an immune system.

Edible Vaccine

Edible Vaccines are prepared by introducing selected desired genes into plants and inducing these genetically modified plants to manufacture the encoded proteins. This process is known as "transformation" and the altered plants are called "transgenic plants" [2-3].

Creating edible vaccines involves introduction of selected desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. This process is known as "transformation," and

the altered plants are called "transgenic plants." Like conventional subunit vaccines, edible vaccines are composed of antigenic proteins and are devoid of pathogenic genes [4]. Thus, they have no way of establishing infection, assuring its safety, especially in immunocompromised patients. Conventional subunit vaccines are expensive and technology-intensive, need purification, require refrigeration and produce poor mucosal response. In contrast, edible vaccines would enhance compliance, especially in children, and because of oral administration, would eliminate the need for trained medical personnel. Their production is highly efficient and can be easily scaled up. For example, hepatitis-B antigen required to vaccinate whole of China annually, could be grown on a 40-acre plot and all babies in the world each year on just 200 acres of land [5-6]. They are cheaper, sidestepping demands for purification (single dose of hepatitis-B vaccine would cost approximately 23 paise), grown locally using standard methods and do not require capital-intensive pharmaceutical manufacturing facilities. Mass-indefinite production would also decrease dependence on foreign supply. They exhibit good genetic stability. They are heat-stable; do not require cold-chain maintenance; can be stored near the site of use, eliminating long-distance transportation. Non-requirement of syringes and needles also decreases chances of infection. Fear of contamination with animal viruses - like the mad cow disease, which is a threat in vaccines manufactured from cultured mammalian cells - is eliminated, because plant viruses do not infect humans.

Edible vaccines activate both mucosal and systemic immunity, as they come in contact with the digestive tract lining. This dual effect would provide first-line defense against pathogens invading through mucosa, like Mycobacterium tuberculosis and agents causing diarrhea, pneumonia, STDs, HIV, etc. Scientists place high priority on combating the diarrheal agents - Norwalk virus, Rotavirus, Vibrio cholera and enterotoxigenic *E.* coli (ETEC) -responsible for about three million infant deaths/year, mainly in developing countries [7-9]. Administration of edible vaccines to mothers might be successful in immunizing the fetus-in-utero by transplacental transfer of maternal antibodies or the infant through breast milk. Edible vaccines seroconvert even in the presence of maternal antibodies, thus having a potential role in protecting infants against diseases like group-B Streptococcus, respiratory syncytial virus (RSV), etc., which are under investigation. Edible vaccines would also be suitable against neglected/rare diseases like dengue, hookworm, rabies, etc. They may be integrated with other vaccine approaches, and multiple antigens may also be delivered. Various foods under study are banana, potato, tomato, lettuce, rice, etc.[10] Edible vaccines are currently being developed for a number of human and animal diseases, including measles, cholera, foot and mouth disease and hepatitis B, C and E.

Need and Importance of Edible Vaccines

The World Health Organization (WHO) has called for the development of new strategies to deliver vaccines. WHO estimates that 10 million children die in developing countries each year from infectious diseases that could be prevented with vaccines the masses of poor and developing countries. That would improve access to basic vaccines and give struggling agricultural economies a shot in the arm. Conventional injectable vaccines are expensive and require a semi-skilled person for administration with needles that are not easily available everywhere in developing countries. Hepatitis B and C and HIV can be transmitted very easily by deliberate or accidental reuse of needles. Further, the vaccine vials have to be stored under refrigerated conditions to maintain their stability. Hence we need cost-effective, easy-to-administer, easy-to-store, fail-safe and socio-culturally readily acceptable vaccines and their delivery systems. Plants, plant viruses and animals, especially mammals can be genetically engineered to produce vaccines against pathogens which primarily enter through oral route.

Production of edible vaccines utilizes combination of inventions in medical and plant sciences to create effective and affordable pharmaceutical products. They are easy to administer and show better compliance, especially in children and do not require trained medical personnel. A large number of efficient, large scale production processes are being developed for them. They are administered without purification, reducing production cost and can be easily grown locally without requirement of capital intensive pharmaceutical manufacturing facilities .Edible vaccines show appreciable level of genetic and thermal stability eliminating the need of maintenance of refrigerated conditions during transportation and storage.

Mechanism of Action

Plant parts are fed directly since the outer tough wall of plant cells acts to protect the antigens against attack by enzymes, gastric and intestinal secretions. This method is known as bio-encapsulation. The plant cell wall breaks in the intestines to release the antigens. The antigens which are released are taken up by M cells in the intestinal lining that are present over the Payer's patches (in the ileum) and the gut associated lymphoid tissue (GALT). Peyer's patches refer to the groups of lymphatic nodules also called aggregated lymphatic follicles. The antigens are then passed onto macrophages and other APCs (antigen presenting cells) and local lymphocytes. This triggers formation of serum IgG,IgE and local IgA antibodies and memory cells. These immediately neutralize the infectious agent present in the body [12-13].

Edible vaccines elicit both mucosal and systemic immune response. Plant parts are fed directly the vaccine delivered through food is released directly in the intestine, the site of absorption of proteins. The vaccine is protected from gastric digestion due to bio - encapsulation into plant cells with pectic and cellulosic cell walls that are degraded due to enzymatic action of intestinal microflora. The vaccine delivered through food is released directly in the intestine, the site of absorption of proteins. The vaccine is protected from gastric digestion due to bio - encapsulation into plant cells with pectic and cellulosic cell walls that are degraded due to enzymatic action of intestinal microflora.

Once the vaccine is released in the intestine, it is collected by M cells of epithelium, passed on to the antigen presenting cells (APCs) that process and present the antigen to T and B cells in the payer's patches. The activated immune cells travel to lymph nodes for clonal amplification, plasma cell generation and distribution to other mucosal surfaces. The plasma cells secret IgA antibodies at mucosal surfaces that are involved in neutralization of antigens that enter through oral route. Some of the activated B cells enter systemic circulation and transform into plasma cells secreting IgG, imparting systemic immunity.

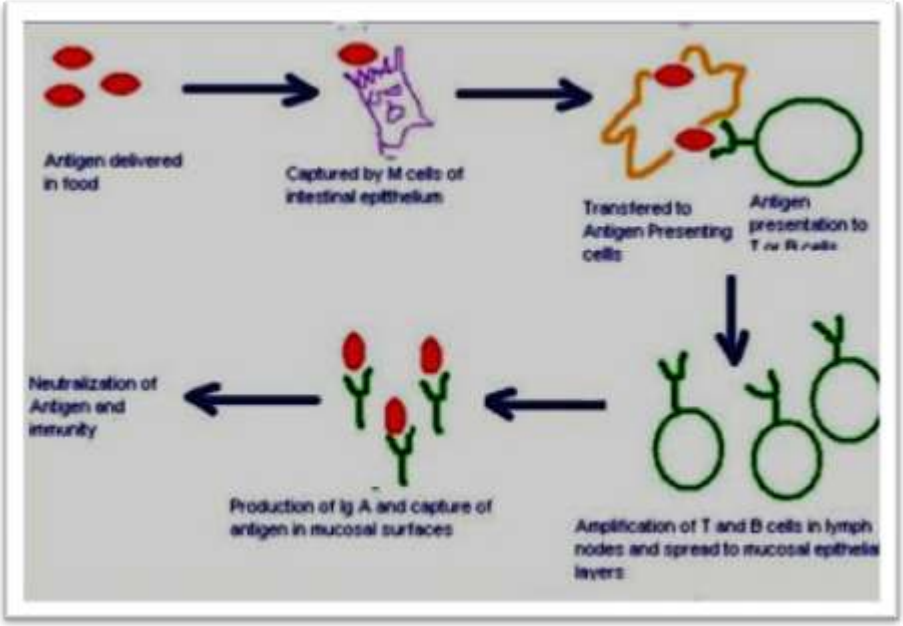

Fig-1: Mode of action of edible vaccines

Preparation of Edible Vaccines

Introduction of foreign DNA into plant's genome can either be done by bombarding embryonic suspension cell cultures using gene-gun or more commonly through Agrobacterium tumefaciens, a naturally occurring soil bacterium, which has the ability to get into plants through some kind of wound (scratch, etc.). It possesses a circular "Ti plasmid" (tumor inducing), which enables it to infect plant cells, integrate into their genome and produce a hollow tumor (crown gall tumor), where it can live. This ability can be exploited to insert foreign DNA into plant genome. But prior to this, the plasmid needs to be disarmed by deleting the genes for auxin and cytokinin synthesis, so that it does not produce tumor. Genes for antibiotic resistance are used to select out the transformed cells and whole plants, which contain the foreign gene; and for expressing the desired product, which can then be regenerated from them.The DNA integrates randomly into plant genome, resulting in a different antigen expression level for each independent line,[10] so that 50-100 plants are transformed together at a time, from which one can choose the plant expressing the highest levels of antigen and least number of adverse effects. Production of transgenic plants is species dependent and takes 3-9 months. Reducing this time to 6-8 weeks is currently under investigation. Some antigens, like viral capsid proteins, have to self-assemble into VLPs (virus- like particles). VLPs mimic the virus without carrying DNA or RNA and therefore are not infectious. Each single antigen expressed in plants must be tested for its proper assembly and can be veriÞ ed by animal studies, Western blot; and quantified by enzyme-linked immunosorbent assay (ELISA). [11, 14].

Second Generation of Edible Vaccines:

Successful expression of foreign genes in plant cells and/or its edible portions has given a potential to explore further and expand the possibility of developing plants expressing more than one antigenic protein. Multi-component vaccines can be obtained by crossing two plant or animal lines harboring different antigens. B subunit of *Vibrio cholerae* toxin (VC-B) tends to associate with its copies of itself, forming a doughnut-shaped five-member ring with a hole in the middle. Using this strategy, several different antigens can be

exposed to immune cells simultaneously, for example, a trivalent edible vaccine can be developed that shall be effective against cholera, enterotoxigenic *E. coli* and rotavirus. This shall be socio-culturally a most-welcome approach. Global alliance for vaccines and immunization (GAVI) gives very high priority to such combination vaccines for developing countries[9, 15].

Limitations in Administration of Edible Vaccines

Optimization of dose of vaccine:

The success of immunization depends on administration of antigen in sufficient dose which has to be decided on the basis of age, weight, and physiological state of the person. A low dose may not be sufficiently immunogenic while high doses may cause tolerance. The dose of edible vaccine shall vary with the fruit, plant, size, ripeness and protein content of the edible part. One of the practical difficulties in administration is that children may not eat the edible part completely or may spit it later on. A better method of administration would be to concentrate the vaccine into a baby food powder. Batch to batch consistency, uniformity of dosage and purity are the major regulatory concerns.

Protein content of transgenic organisms:

The immunogenic protein may not accumulate in high concentrations in the transgenic organisms. Furthermore; the immunogenicity of the protein may be lowered. Hence edible vaccines have to be administered in high doses .Attempts to increase the number of copies of the foreign gene leads to deformities in the transgenic organisms. High level expression of transgene can be achieved by linking it to regulatory elements which increase expression of the transgene in specific organs and at appropriate developmental stage of the organism.

Immunogenicity:

Edible vaccines show low immunogenicity. To enhance immunogenicity, mucosal adjutants, better targeted to the immune system, may be used, like molecules that bind to M cells in the intestine lining and pass them to immune cells .A natural adjuvant in the form of non infective subunits of *E. coli* and Cholera toxins can be fused with gene inserts as shown in the figure-2 .

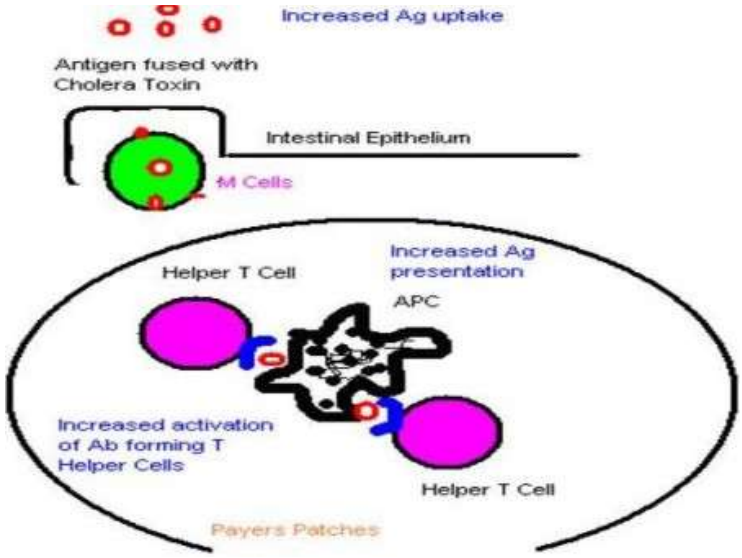

Fig -2: Role of Cholera Toxin as engineered adjuvant for edible vaccine. [8]

Antigenic variability:

It would be difficult to develop edible vaccines against diseases caused by antagonistically variable groups of pathogens as well as those caused by multiple varieties of organisms (dengue) or by complex parts from different life cycles of parasites (malaria) or by rapidly mutating organisms like HIV.

Thermal Stability:

Proteins in food are heat labile. Cooking results in destruction of proteinthe materialshall destroy the immunogenicity nature of the protein. Therefore, care is to should be taken to introduce immunogenic proteins in foods that are eaten raw. Research is on to increase the heat stability of immunogenic proteins as well as percent losses due to cooking. It was shown that transgenic potatoes could be boiled for 3 minutes with only a 50% loss of immunogenic protein.

Lack of Awareness and Funds:

Small companies are undertaking most research as edible vaccines are targeted to markets of developing nations. Large companies are more interested in developing vaccines for farm animals as compared to human beings. Very few private and government funding agencies are supporting research on edible vaccines. Many agencies withdrew support

after worldwide concern against use of genetically modified foods.

Regulatory Issues:

It has to be decided whether edible vaccines would be regulated under food, drugs or agricultural products. Further, it is undecided that licensing shall be required for the antigen or genetically engineered fruit or transgenic seeds. The modified plants require green house segregation and separate bodies that ensure that such plants are not released in the environment by any means. Transgenes may spread by pollen, sucking insects, transfer to soil microbes during plant wounding or breakdown of roots and may pollute surface and ground water. Ethical considerations usually restrict clinical trials from directly assessing protection in humans.

Future of Edible Vaccines:

The future of edible vaccines depends on following factors: Socio-cultural acceptability of genetically modified plants; genetic stability of transgenic varieties; proper segregation of transgenic plants and prevention of environment contamination and prevention of potent side effects of transgenes as production of allergens. Vaccines have been successful in creating better health for society during 20th century. Advancing technology, such as oral DNA vaccines, intranasal delivery and edible plant derived vaccines, may lead to a future of safer and more effective immunization. Edible vaccines can be safe and effective modes of immunization. They are better as compared to the traditional vaccines when mass production, distribution and delivery are concerned. However we have to overcome various other technical hurdles before implementing them as routine immunization methods. We hope for development of a cost effective, efficient and safe delivery system for diseases like AIDS, malaria, various intestinal disorders that affect the masses worldwide and edible vaccines shall one day become an un-separable part of global disease prevention strategy.[16-18].

Conclusion

Edible vaccine is a mile stone on the raad to creating inexpensive vaccines that might be particularly useful in immunizing people. Edible plant-derived vaccine may lead to a future of safer and more effective immunization. They would overcome some of the difficulties associated with traditional vaccines, like production, distribution and delivery, and they can be incorporated into the immunization plans. They have passed the major hurdles in the path of an emerging vaccine technology. Before becoming a reality, the technical obstacles, though all seem surmountable, need to be overcome. However, with limited access to essential health care in much of the world and with the scientific community still struggling with complex diseases like HIV, malaria, etc., a cost-effective, safe and officious delivery system in the form of edible vaccines will become an essential component in our disease-prevention arsenal.

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