



EDIBLE VACCINE: A BETTER WAY FOR IMMUNIZATION

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ABSTRACT

Vaccines are used most widely in the world. They have reduced mortality rate caused by various infectious organisms. Their use has been considered one of the safe and effective measure and to control various infectious diseases. But a mind disturbing reality, which has been generally unrecognized, is the ever growing number of individuals suffering from adverse reactions to vaccines. Edible vaccine can be one of the alternatives of the traditional vaccines as they can overcome all the problems associated with traditional vaccines. For obtaining edible vaccine, selected gene of interest is administered to the plant. Transgenic plant is then induced to manufacture the encoded proteins. Edible vaccines are targeted to provide mucosal as well as systemic immunity. This paper reviews the method of preparation, mode of action, advantages and limitations, applications and clinical trials related to edible vaccines.

Keywords: Edible vaccine, Transgenic plant, Mucosal immunity, Systemic immunity

INTRODUCTION

According to WHO, various diseases are responsible for 80% of illness worldwide and cause more than 20 million deaths annually.¹ Vaccines represent an invaluable contribution in the field of biotechnology as they provide protection against various diseases. Conventional vaccines are made up of live/attenuated vaccine and killed vaccine. Newer approaches have also been made with regard to the use of purified antigen vaccines and recombinant vaccines. Despite the successful immunization program, the mortality rates are increasing every year.²

This may be constraints on vaccine production, distribution and delivery. Moreover, the administration of vaccine is cost effective method of combating spread of infections and epidemics.³ So it is necessary to produce new vaccine that have economic and other advantages over the existing vaccines. An alternative approach is the use of edible vaccines. It has given new and dramatic hope for improved life. Edible vaccines are emerging innovations in medical science and plant biology for the efficacious and affordable pharmaceuticals.^{4,5}

To produce edible vaccine, desired gene of interest is introduced in the selected plants to manufacture proteins encoding for the same gene. This process is accomplished by transformation and the altered plant is called transgenic plant.⁶ Thus the process remains the same as with subunit vaccine preparation because it contains only desired antigen and not the whole pathogen. For production of edible vaccines, it is desirable to select a plant whose products are consumed raw to avoid degradation during cooking. Thus, plants like tomato, banana and cucumbers are generally the plants of choice.

The first report of providing the proof of edible vaccine was published under the international patent cooperation treaty in the year 1990.⁷ It got impetus and holds promise as low cost vaccine production system after the expression of Hepatitis B surface (HBs) antigen in tobacco plants shown by Charles Arntzen and coworkers.⁸ A number of attempt have been made subsequently to express various antigens in plants⁹⁻¹⁵ (Table 1).

Later on, multicomponent vaccine placed under second generation edible vaccine has also given a potential to provide protection against various ailments causing disease. Multicomponent edible vaccines can be obtained by crossing two plant lines harbouring different antigens.¹⁶ Adjuvants may also be co-expressed along with the antigen in the same plant.

Production of edible vaccine

The desired gene which codes for antigen is isolated from the microbes and could be handled in two different ways:

1. Appropriate plant virus is genetically engineered to express the desired peptides/proteins. The recombinant virus is then inoculated into the plant. Large numbers of new plants are grown and chimeric virions are extracted and purified. The resultant plant edible vaccines are utilized for immunological purpose.
2. In another approach, the gene of interest is integrated with plant vector by transformation. A variety of techniques have been used to introduce transgene into plant cell; these could be grouped into following categories:
3. Agrobacterium mediated gene transfer: The appropriate gene construct is inserted into the T-region of a disarmed Ti plasmid of Agrobacterium. The recombinant DNA is placed into Agrobacterium; a plant pathogen which is co-cultured with the plant cells or tissues to be transformed.¹⁷ The drawback of this method is that it gives low yield and the process is slow. This method workd especially well for dicotyledenous plants like potato, tomato and tobacco. Studies have also proved that the genes are expressed by this method in experimental animals and plants.¹⁸⁻²⁰
4. Biolistic method: The gene containing DNA coated metal (e.g. gold, tungsten) particles are fired at the plant cells using gene gun.²¹ Those plant cells that take up the DNA are then allowed to grow in new plants, and are cloned to produce large number of genetically identical crop. This method is quite attractive because DNA can be delivered into cells of plant which makes gene transfer independent of regeneration ability of the species. But the chief limitation is the need for costly device particle gun.
5. Electroporation: Here there is introduction of DNA into cells by exposing them for brief period to high voltage electrical pulse which is thought to induce transient pores in the plasma lemma.²² The cell wall presents an effective barrier to DNA therefore, it has to be weakened by mild enzymatic treatment so as to allow the entry of DNA into cell cytoplasm.
6. Mode of action
7. The first line of the defense mechanism is mucosal immunity. Vast majority of human pathogens initiates infections at

mucosal surfaces, by making gastrointestinal, urogenital and respiratory tracts as major routes of entry into the body.²³

8. The most effective route of mucosal immunization is oral route. The oral vaccine can generate mucosal immunity, antibody mediated immune response and cell mediated immune response. The antigen containing plant vaccine when introduced orally does not get degraded by gastric enzymes because of the presence of tough outer wall of plant cell. Release of antigen and its breakdown from plant vaccine occurs near Payer's patches of intestine.²⁴ The released antigen is taken up by the M-cells and is presented to B cells with the help of antigen presenting cells (APC). The activated B-cells get differentiated into plasma cells and secrete Ig A class of antibody and elicit mucosal immunity and humoral immunity. Another important component of mucosal immunity is T-cell mediated immune response where the T-cells specifically recognize pathogens and directly kill the invader themselves. It also helps indirectly to the antibodies to clear infection. T-cells produced in the mucous are capable of travelling the mucosal tissues through special 'homing' receptors on their membranes. This means that if an immune response is generated in gastrointestinal lining, T-cells produced there can travel to other mucosal sites, (e.g. the lungs, nasal cavity) providing protection over a large surface area.

Advantages of edible vaccine

1. Edible vaccines are effective as delivery vehicle for immunization because adjuvants which enhance the immune response are not necessary.
2. Edible vaccine can elicit mucosal immunity which is not observed in traditional vaccines
3. Edible vaccines are also cost effective in storage, preparation, production and transportation. Vaccines produced by biotechnological method are stable at room temperature, unlike traditional vaccine which need cold chain storage which increases the yearly cost to preserve vaccines.²⁵ Moreover, the seeds of transgenic plants could be dried as there is less moisture content in seeds and the plants with oil or their aqueous extracts possess more storage opportunities.²⁶ For production of edible vaccines, costly equipments and machines are not necessary as they could be easily made on soil rich motherland and the cost for growing plants is also low compared to cell culture grown in fermenters.
4. As edible vaccines are produced from plants, they are easily available. Manufacturing cost is low as there is no need of special premises for manufacturing them and facilities like sterilization as required in traditional vaccines.
5. Edible vaccines are easily acceptable as they do not require administration by injection unlike traditional vaccines. Thus there is also reduced need of medical personnel and the contamination risk is low as sterile conditions are not necessary.¹⁷ The risk of spreading environmental contamination by spreading second hand diseases is minimized.
6. Plant derived vaccines could be the source for new vaccines combining numerous antigens. These multicomponent vaccines are called second generation vaccines as they allow for several antigens to approach M cells simultaneously.
7. An edible vaccine improves the safety of individual as compared to traditional vaccine since there is no possibility of proteins to reform into infectious organism. Edible vaccines are subunit preparation and do not involve attenuated pathogens.
8. Edible vaccine is easy for mass production system by breeding as compared to an animal system.
9. Limitations/Concerns of Edible vaccines
10. Edible vaccine is easy to access but still it leaves some challenges. The major problems associated with edible vaccine are as follows:

11. Possibility of development of immunotolerance to the vaccine protein or peptide.
12. Consistency of dosage form differs from plant to plant and generation to generation, protein content, patient's age, weight, ripeness of the fruit and quantity of the food eaten in absence of availability of methods for standardization of plant material/product. Low doses of the edible vaccine if consumed produces less number of antibodies and high doses causes immune tolerance.
13. Stability of vaccine differs from plant to plant.
14. Some food cannot be eaten raw (e.g. potato) and needs cooking which will denature or weaken the protein present in it.²⁷
15. Variable conditions for edible vaccine are also a major problem. Potatoes containing vaccine to be stored at 4°C and could be stored for longer time while a tomato does not last long. Thus these vaccines need to be properly stored to avoid infection through microbial spoilage.
16. Another concern regarding edible vaccine is need of proper distinguishing characters to identify between 'vaccine fruit' and 'normal fruit' to avoid misadministration of vaccine which could lead to tolerance.
17. Glycosylation pattern of plants and humans is different which could affect the functions of vaccines.

Applications of edible vaccines

Autoimmune diseases: Investigators have identified several cell proteins that can elicit autoimmunity among the people suffering from Type I diabetes. An attempt was made for the development of plant based diabetes vaccines in potatoes and tobacco containing insulin linked to the innocuous B subunit of the V. cholera toxin to enhance uptake of the antigens by M cells. The transgenic potato and tobacco plants when fed to non obese diabetic mice exhibited increased levels of IgG, an antibody associated with cytokines that suppress harmful immune responses. Thus feeding of the vaccines to mouse that becomes diabetic helps in suppressing the autoimmune attack and may delay the rise in blood sugar level. ²⁸⁻²⁹

Gastrointestinal disorders: WHO have demonstrated that cholera vaccine can provide cross protection against enterotoxigenic E. coli heat labile enterotoxin (LT-B). Transgenic potatoes expressing LT-B were fed to mice and was found to induce both secretory and serum antibodies. Later on evidence were also provided which showed that cooking raw potatoes does not inactivate the antigen present in edible vaccine. Thus, the spectrum of plants for producing edible vaccine may be expanded beyond raw food plants such as fruits.³⁰⁻³²

Malaria: To develop malaria vaccine three antigens were used namely merozoite surface protein (MSP) 4, MSP 5 and MSP4/5. When mice were orally immunized with this recombinant antigen and co administered with CTB as mucosal adjuvant, induced immune response. However oral delivery of plant derived malaria vaccine inducing immune response is uncertain.

Measles: Currently available vaccine for measles is live attenuated vaccine having certain disadvantages. For preparation of edible vaccine, MV-H antigen was selected and introduced in tobacco plant with the help of plasmid vector. It was found that serum antibodies were inducing immune response against antigen. It was also found that fecal samples of immunized animal with antigen containing IgA antibodies.

Some other therapeutic uses and applications of edible vaccine is shown in Table 2.

Safety aspects

The main aspects for safety are plant/crops (food) contamination through cross pollination and of vaccine itself in plant debris spreading dust and other pollutant in surfaces and ground waters. The vaccine antigen may affect browsing animals and humans living in the area drinking vaccine polluted water or breathing vaccine polluted dust. The cultivation and production of pharmaceutical

crops should be limited to control the production facilities like greenhouse, or in plant tissue culture, that prevent the environmental release of biopharmaceuticals.

Clinical trials

Successful expression of antigen in plant has been demonstrated in the past.⁴¹⁻⁴³ Various transgenic animal feeds are currently undergoing clinical trials in various animals.^{44,45} The vaccines have

also been checked for their efficacy in humans.⁴⁶⁻⁴⁸ Although it is future vaccine, still some challenges are yet to be overcome before edible vaccine can become reality. Different selected patents on edible vaccine exist (Table 3).

CONCLUSION

Edible vaccine might be solution to get rid of various ailments as it has more advantages compared to traditional vaccine. It would

Table 1: Examples of edible vaccines

Name of the vaccine	Vector	Pathological conditions	Etiological agent	Reference
Rabies	Tobacco	Rabies	Rabies virus	9,10
Hepatitis B	Potato, Tobacco	Hepatitis B	Hepatitis B virus	11
Gastroenteritis	Maize	Gastroenteritis	Corona virus	12,13
HIV	Tomato	AIDS	HIV virus	14
Cholera	Potato	Cholera	Vibrio cholera	15

Table 2: Applications of edible vaccines

Plants	Applications	Reference
Wheat, Rice	Treatment of cancer	33,34
N. tabacum	Dental caries	28,35
Soyabean	Herpes	37
T. benthamiana	Colon cancer	38
Hepatitis virus	Hepatitis	39,40

Table 3: Some patents of edible vaccines

Patent holder	Claim	Reference
Ribozyme pharm	Nucleic acid vaccine used to treat or prevent viral infections in plants, animals or bacteria	49
University Loma Linda	Gene constructs used to produce edible vaccines to treat autoimmune diseases, including diabetes and multiple sclerosis	49
Rubicon Lab	Retrovirus expressed in animal or plant cells useful as virus and cancer vaccines	50,51
Biosource	Plant viral vector with potential as anti-HIV vaccine; recombinant proteins for use in vaccine to protect against parasitic infection	49,52
University of Texas	Hepatitis B core antigen recombinant vaccine	51,53
University of Yale	Vaccine against invertebrates	50
Institute of Pasteur	Attenuated E. coli vaccine for use in gene therapy	49

overcome the problems associated with traditional vaccine like cost, production, distribution and delivery and could be incorporated into the immunization plans. It would be more beneficial and profitable to populations of developing world. But still there is lack of production and investment in this new technology but it will be likely conquered to make plant derived vaccine more efficient and dependable.

REFERENCES

- Jogdand S N. Medical Biotechnology. Himalaya publishing house. Mumbai, 2000.
- Landridge W. Edible vaccines. Scientific Am 2000;283:66-71.
- Lal P, Ramachandran V G, Goyal R. Sharma R. Edible vaccines: Current status and future. 2007;25:93-102.
- Morr T S, Gomez Lim M A, Palmer K E. Edible vaccines: A concept comes of age. Trends Microbiol 1998;6:449-453.
- Daniell H, Streatfield S J, Wyckoff K. Medical molecular farming: Production of antibiotics, biopharmaceutical and edible vaccine in plants. Trends Plant Sci 2001;6:219-226.
- Sibila J, Snjezana M, Natasa B. Production of biopharmaceuticals, antibodies and edible vaccines in transgenic plants. Current studies of biotechnology. 121-127
- Mason H S, Arntzen C J. Transgenic plant as vaccine production system. Trends Biotechnol 1995;13:388-92.
- Mason H S, Lam D M K, Arntzen C J. Expression of hepatitis B surface antigen in transgenic plants. Proc Natl Acad Sci USA 1992;89:11745-49.
- Hooper D C, Pierarrd L, Modelska A, Otvos L J, Fu Z F et al. Rabies ribonucleocapsid as an oral immunogen and

- immunological enhancer. Proc Natl Acad Sci USA 1994;91:10908-912.
- Mason H S, Ball J M, Shi J J. et al. Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice. Proc Natl Acad Sci USA 1996;93:5335-40.
- Tacket C O, Mason H S, Losonsky G. et al. Human immune responses to a novel norwalk virus vaccine delivered in transgenic potatoes. J Infect Dis. 2000;182:302-5.
- Tuboly T, Yu W, Bailey A. et al. Immunogenicity of porcine transmissible gastroenteritis virus spike protein expressed in plants. Vaccine 2000;18:2023-8.
- Castanon S, Marin M S, Martin-Alonso J M. et al. Immunization with potato plants expressing VP60 protein protects against rabbit hemorrhagic disease virus. J Virol 1999;73:4452-5.
- http://www.istc.ru
- Arakawa T, Chong D K, Merritt J L. et al. Expression of cholera toxin B subunit oligomers in transgenic potato plants. Transgenic Res 1997;6:403-13.
- Jie Yu, William H.R. Langridge. A plant-based multicomponent vaccine protects mice from enteric diseases. Nature Biotechnology 2001;19:548-552.
- Streatfield S J. Mucosal immunization using recombinant plant based oral vaccines. Methods 2006;38:150-57.
- Mariotti D, Faontana G S, Santin. Genetic transformation of grain legumes: Phaseolus vulgaris L. and P. coccineus. J Genetic Breed 1989;43:77-82.
- Mercenier A, Wieddermann U, Breitender H. Edible genetically modified microorganisms and plants for improved health. Curr Opin Biotechnol 2001;12:510-515.
- Chikwamba R, Cunnick J, Hathway D, McMurry J, Mason H et al. A functional antigen in a practical crop: LT-B producing maize

- protects mice against E.coli heat labile enterotoxin (LT) and chorea toxin (CT). *Transgenic Res* 2002;11:479-493.
21. Taylor N J, Fauquet C M. Microparticle bombardment as a tool in plant science and agricultural biotechnology. *DNA Cell Biol* 2002;21:963-977.
 22. Singh B D. *Biotechnology*. Kalyani publishers. New Delhi. 2002:323.
 23. Tacket C O, Mason H S. A review of oral vaccination with transgenic vegetables. *Microbes Infect* 1999;1:777-783.
 24. Rudzik R, Claney R L, Perey Y E. et al. Repopulation with IgA containing cells of bronchial and intestinal lamina propria after transfer of homologous payer's patch and bronchial lymphocyte. *J Immunol* 1975;144:1599.
 25. Nochi T, Takagi H, Yuki Y. et al. Rice based mucosal vaccine as a global strategy for cold chain and needle free vaccination. *Proc Natl Acad Sci USA* 2007;104:10986-10991.
 26. Pascual D W. Vaccines are for dinner. *Proc Natl Acad Sci USA* 104;10757-10758.
 27. Moss W J, Cutts F, Griffin D E. Implications of human immunodeficiency eradications of measles. *Infect Dis* 1999;29: 4452-4455.
 28. Ma J K C, Hiatt A, Hein M. et al. Generation and assembly of secretory antibodies in plants. *Sciences* 1995;268:716-719.
 29. Travis J. Scientists harvest antibodies from plants. *Science News* 1998;5:359.
 30. Mason H S, Haq F A, Clement J D. et al. Edible vaccine protects mice against Escherichia Coli het labile enterotoxin (LT): Potatoes expressing a synthetic LT-B Gene. *Vaccine* 1998;16:1336-1343.
 31. Leben M, Johansson S, Osek J. et al. Large-scale production of Vibrio cholera toxin B subunit for use in oral vaccines. *Bio/Technology*. 1993;11: 1574-1578.
 32. Richter L, Mason H S, Arntzen C J. Transgenic plants created for oral immunization against diarrhoeal diseases. *Travel Med* 1996;3:52-56.
 33. Torres E, Vaquero C, Nicholson L. et al. Rice cell culture as an alternative production system for functional diagnostic and therapeutic antibodies. *Transgenic Res* 1999;8:441-449.
 34. Vaquero C, Sack M, Chandler J. et al. Transient expression of a tumour-specific single-chain fragment and a chimeric antibody in tobacco leaves. *Proc Natl Acad Sci U S* 1999;96:11128-11133.
 35. Ma J K C, Hikmat B Y, Wycoff K. et al. Characterization of recombinant plant monoclonal secretory antibodies in preventing immunotherapy in human. *Nat Med* 1998;4:601-606.
 36. McCormick A, Kumagai M, Hanley K, Turpen T, Hakim I et al. Rapid production of specific vaccines for lymphoma by expression of the tumour-delivered single-chain Fv epitopes in tobacco plants. *Proc Natl Acad Sci U S* 1999;96:703-708.
 37. Zeitlin L, Olmsted S S, Monech T R, Co M S, Martinell B J et al. A humanized monoclonal antibody in plants using a plant virus vector. *J Immunol Methods* 1998;220: 69-75.
 38. Verch T, Yusibov V, Koprowski H, Expression and assembly of a full-length monoclonal antibody in plants using a plant virus vector. *J Immunol Methods* 1998;220: 69-75.
 39. Domansky N, Organ-specific expression of hepatitis B surface antigen in potato. *Biotech Lett*, 1995;17:863-866.
 40. Richter L J, Thanavala Y, Arntzen C J. et al. Production of hepatitis B surface antigen in transgenic plants for oral immunization. *Nat Biotechnol* 2000;18:1167-1171.
 41. Tripurani S K, Reddy N S, Sambasiva Rao K R. Green revolution vaccines, edible vaccines. *Afr J Biotechnol* 2003; 2: 679-83.
 42. Mason H S, Lam D M, Arntzen C J. Expression of hepatitis B surface antigen in transgenic plants. *Proc Natl Acad Sci USA* 1992; 89 :11745-9.
 43. Ehsani P, Khabiri A, Domansky N N. Polypeptides of hepatitis B surface antigen in transgenic plants. *Proc Natl Acad Sci USA* 1997, 190:107-111.
 44. <http://www.sciencedaily.com/releases/1999/09/990913145730.htm>.
 45. http://www.checkbiotech.org/blocks/dsp_document.cfm?doc_id=3547.
 46. Tacket C O, Mason H S, Losonky G. et al. Wasserman S et al. Immunogenicity in human of a recombinant bacterial antigen delivered in transgenic potato. *Nat Med* 1998;4: 607-609.
 47. Tacket C O, Mason H S, Losonky B E. et al. Human immune responses to a novel Norwalk virus vaccine delivered in transgenic potatoes. *J Infect Dis* 2000;182:302-305.
 48. Kong Q, Richter I, Yang Y F. et al. Oral immunization with hepatitis B surface antigen expressed in transgenic plants. *Proc Natl Acad Sci USA* 2002; 11539-11544.
 49. www.prodigene.com
 50. Khoudi H, Laberge S, Ferullo. et al. Production of a diagnostic monoclonal antibody in perennial alfalfa plants. *Biotechnol Bioeng* 1999;64:135-143.
 51. Toonen J. Seeds of a new medicine. *Biotechnology and development monitor* 1996;27:12-14.
 52. WHO and UNICEF. The state of the world's vaccines and immunization. <http://www.unicef.org/newsline/vpressr.htm>. 1996.
 53. A Pollack. Ventures aim to put farm in pharmaceutical vanguard. *The New York Times* May 14. 2000.