



EDIBLE VACCINES-NEW TECHNOLOGY TO COMBAT DISEASES

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ARTICLE INFO

Article History:

Received 4th January, 2019

Received in revised form 25th

February, 2019

Accepted 18th March, 2019

Published online 28th April, 2019

Key words:

Edible, vaccines, infectious diseases

ABSTRACT

Edible vaccines are cost-effective, socially acceptable, easy to use with no storage problem. It involves selection and introduction of desired genes in plants for manufacture of encoded proteins. Foods which are mainly used as carrier/vector include fruits (banana), cereals (rice, wheat, corn) and vegetables (tomatoes, potatoes, lettuce). In addition to reducing incidence of diseases like hepatitis, diarrhea along with other infectious diseases, edible vaccines also have role in preventing autoimmune diseases, cancer therapy, birth control etc. The future of edible vaccines depends on resistance power to genetically modified foods. The pros and cons of this emerging vaccine technology have been discussed in this review which needs to be understood before application on large scale.

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INTRODUCTION

Usually vaccination program is a failure which results in untimely death of infants before they can celebrate their first birthday. In 1990, Arntzen developed the concept of edible vaccines. Poultry vaccine which was developed by Dow AgroSciences was the first product though earlier tobacco was used to produce surface antigen for preventing dental caries from *Streptococcus mutans*^[1]. Edible vaccines are transgenic plant/animal based products (pharmaceuticals) which trigger immune response^[2]. Through transformation, transgenic plants are produced. Edible vaccines consist of antigenic protein and possess genetic stability being heat stable and are easy to consume requiring no trained personnel and transportation facilities. On oral consumption these edible vaccines stimulate mucosal and systemic immunity which act as defence mechanism for pathogens (*Mycobacterium tuberculosis*, Norwalk virus, *Vibrio cholerae*, Rotavirus, enterotoxigenic *E. coli*) causing diarrhea, STDs, pneumonia, HIV, etc. Many of placental infections transferred from mother to child through breast milk may be prevented by using edible vaccine. Moreover, use of edible vaccine can help in preventing diseases like hookworm, rabies, dengue etc. Trials are in pipeline in developing edible vaccines against measles, foot and mouth disease, hepatitis (B, C and E).

Developing an Edible Vaccine: Gene which is selected from microbes for encoding specific antigen can be produced in two ways:

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Using genetic engineering, desired peptides/proteins are produced using suitable plant virus; later this recombinant virus is incorporated into plant to obtain new plants from which chimeric virions are purified after isolation. This plant vaccine is used for immunological applications.

In second method, using transformation, desired gene is incorporated with plant vector.

Other Methods Include

Agrobacterium mediated gene transfer: This method includes recombinant DNA incorporation in T-region of disarmed Ti plasmid of plant pathogen (*Agrobacterium*) and co-culturing with plant cells/tissues which are to be transformed. It is a slow process giving low yield but successful in dicotyledonous plants (tomato, potato, tobacco)^[3,4].

Biolistic method: It is expensive and sophisticated method as it involves use of gene gun for firing genes with DNA coated metal (tungsten, gold) particles at plant cells which grow into new plants. These are later cloned to produce sufficient amount of crops having same genetic composition^[5,6].

Electroporation: DNA is inserted into plant cell and exposed to electrical pulse of high voltage which results in transient pores in plasma lemma^[7].

Mechanism of Action: Mucosal immunity is of prime importance which can be attained by oral vaccines which also can produce antibody mediated immune response along with cell-mediated immune response^[8]. Due to cell wall, plant vaccines easily do not get hydrolysed by enzymatic secretions in body. Antigens are released in intestine to be taken up by M cells which further are passed to macrophages, lymphocytes and as a response, serum Ig G, Ig A, Ig E and memory cells.

Oral/edible vaccines actually contain DNA fragments which code for surface protein of the original pathogen.

Some Examples of Edible Plant Vaccines

Banana vaccines: In the coming days, it is expected that one can get rid of problems like hepatitis B or cholera by intake of banana. Although scientists have got success in vaccine production using engineered potatoes, carrot, brinjal, lettuce, rice, tobacco^[9] but use of banana may bring revolution as it is a tropical crop, virus genetic material forms quickly part of plant, needs no cooking before consumption by people in developing countries. Only drawback with banana to be used as vector is that it requires 2-3 years to mature and after ripening spoils at rapid rate^[10]. Hepatitis B virus attacks liver causing cancer. When single gene is transferred into plants like tomato, banana it multiplies and reproduces thousands of it within fruit. On consumption as banana chip or paste of tomato in wafer, this protein enters blood stream through the intestine and produces antibodies against hepatitis B. In 1990, E-coli antibodies were genetically introduced in potato which were fed to mice and human beings. Raw potato, tomato was effective compared to cooked potato in which antibodies producing proteins were unstable.

Brinjal: Research is in process to use brinjal as vector for production of vaccines, as it also has additional advantages like low calories, richness of fibre, presence of anthocyanins, nasunin.

Maize: These plants are being used to produce protein for preparation of Hepatitis B virus vaccine. The main advantage in using them is that these are cheap, require no refrigeration, skilled person and needles for delivery of vaccine. But cooking is required which adds to problem in its application.

Rice: Since rice forms staple crop for many countries of world, it is being used as a research material for development of vaccine^[11]. Genetically modified rice is being used for production of vaccine against cholera which can be stored for more than one year requiring no purification or refrigeration^[12]. The oral immunization with such transgenic rice which codes for cholera toxin B sub unit (CTB) is unable to stimulate serum Ig G response against storage protein of rice. As CTB expresses in endosperm, it is resistant to gut secretions. Transgenic rice which encodes fusion protein using glycinin AlaB1b (soybean seed storage protein) and allergen peptides (Japanese cedar pollen) has been produced. Cooking does not affect the properties of such transgenic rice which is also able to treat autoimmune diseases. Maize derived heat labile enterotoxin B subunit when fed to nine human volunteers in three 1.0 mg doses, only in seven individuals serum Ig G response was produced^[13]. When soybean (having 35-40% Protein) was used, 2.4% of total seed protein was having heat labile enterotoxin B. Further when this soybean was fed to mice, IgG and IgA anti-LTB antibody response was noted which can help in protection against diarrhea.

Potato: Trials have indicated that hepatitis B protein providing immune response can be produced in potato. When mice were fed raw potato with hepatitis B surface antigen in three doses along with cholera toxin, antibodies developed in mice against hepatitis B after three weeks but it declined within weeks. Once commercial vaccine was injected, it activated the memory cells, so antibodies level again elevated. Polyadenylation signal stabilizes messenger RNA which is

responsible for protein translation. The edible Vaccines have been tested against pathogenic forms of E.Coli. and Norwalk virus usually spread by contaminated food^[14,15]. These vaccines are affordable, easy to use and effective but the only disadvantage is decrease in immunogenicity due to cooking.

Tomato: Possibility has been searched for use of tomato as vector for producing vaccines against rabies, anthrax, HIV/AIDS, malaria^[16]. Transgenic tomatoes have been found effective against Norwalk virus^[17] and Hepatitis B. Surface protein which is specific to the virus has been produced in tomato which help in developing immune response on consumption in individual. Tomato can be grown quickly and cultivated on large scale but is perishable. Rabies glycoproteins have been expressed in spinach^[18] and hepatitis B surface antigen in lettuce^[19,20].

Advantages of Edible Vaccines: Edible vaccines possess efficient action mode for immunization, mostly mucosal immunity. These are cost-effective (no cold storage^[21], trained personnel, purification or needles required), eco-friendly and easy to use. The transgenic plants in form of pills, chips, puddings can be prepared to attract consumers. Since plant are used the seeds of these can be stored easily. If these transgenic plants contain oil or are in form of aqueous extract^[22], then possible to store for longer period. Edible vaccines lack heat killed pathogens and can be produced on large scale by breeding. If produced in native crop by engineering, then there is no need for transportation and distribution as these would be consumed locally.

Disadvantages of Edible Vaccines: It is still doubtful whether the antigens in edible vaccine will trigger immune system in right direction. Vaccine dose is still not standardized and is affected by various factors like plant, generation, ripeness of fruit, amount of transgenic material consumed, age and weight of consumer, allergic reactions and nature of glycosylation (whether in plant or human being)^[23]. In future resistance problems may come in force. Moreover continuous changes occurring in plant and microbial infestation may affect vaccine production.

Other Applications of Edible Vaccine

Cancer therapy: Monoclonal antibodies in plants like soybean (BR-96) attack doxorubicin which is mainly considered as cause of cancer in breast, ovary, colon or lung^[24].

Birth control: Tobacco Mosaic Virus administration results in protein found in mouse zoono pellucida (ZB3 protein) which prevents egg fertilization in mice.

Chloroplast transformation: Since chloroplast genome is usually maternally inherited, so on transformation, there is less risk by cross-pollination and more of transgenic protein can be accumulated^[25].

Role in autoimmune diseases: Many of autoimmune disorders like Type 1 diabetes, rheumatoid arthritis, multiple sclerosis, transplant rejection can be prevented^[26].

Recombinant drugs/proteins: Plants by genetic engineering are being used to produce enzymes, (glucocerebrosidase in tobacco), drugs like albumin, interferon and serum protease^[27].

Second-Generation' Edible Vaccines: Possibility is being searched for developing plant which expresses for more than one antigenic protein like edible vaccine against rotavirus, Enterotoxigenic E.Coli and cholera.

Chimeric Viruses: Cowpea mosaic virus, tobacco mosaic virus, alfalfa mosaic virus, potato virus, cauliflower mosaic virus and tomato bushy stunt virus can be redesigned using epicoot or overcoat technology^[28]. Example Enteritis virus injectable vaccine protected minks from clinical diseases.

The Future of Edible Vaccines: Resistance development against genetically modified food may affect use of edible vaccine in future. Quality control is necessary at each step. Side-effects caused due to consumption of edible vaccine need to be kept in mind before use on large scale.

CONCLUSION

Edible plant derived vaccines present immunization programme in safe and efficient manner in future. Most of the limitations associated with traditional vaccine like production followed by distribution and their delivery can be removed by edible vaccines which have successfully passed obstacles in the path of emerging vaccine technology. In future, its use for eradication of more complex diseases like malaria, HIV needs to be done.

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