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A review on edible vaccines: A novel approach to oral immunization as a replacement of conventional vaccines

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Abstract

Edible vaccines are produced by the introduction of selected desired genes into plants and then inducing these altered plants to produce the encoded proteins. This process is known as "transformation" and the altered plants are called "transgenic plants". Basically Edible vaccines are GM crops that provide extra added "immunity" for certain diseases such as Hepatitis B, diarrhea, pneumonia, STDs, HIV etc. Edible vaccines are composed of antigenic proteins and do not contain pathogenic genes. They have no way of forming infection and safety is assured. Edible vaccine is a new approach to oral immunization. This technology might contribute to global vaccine program and might have a dramatic impact on health care in developing countries. Pathogens attack at mucosal surfaces and therefore, it is the most effective site for vaccination. 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 resistance against pathogens entering through mucosa.

Keywords: edible vaccine; transgenic plants; oral immunization; mucosal immune system

Introduction

Edible vaccines are produced by the introduction of selected desired genes into plants and then inducing these altered plants to produce the encoded proteins. This process is known as "transformation" and the altered plants are called "transgenic plants". Edible vaccines are Genetically Modified (GM) crops that provide extra added "immunity" for certain diseases such as Hepatitis B, diarrhea, pneumonia, STDs, HIV etc. and are composed of antigenic proteins and do not contain pathogenic genes. Edible vaccines hold great promise as a cost-effective, easy-to-administer, easy-to-store, fail-safe and socio culturally readily acceptable vaccine delivery system, especially for the poor developing countries [1]. Important advantage of edible vaccines is elimination of contamination with animal viruses-like the mad cow disease, which is a vulnerability in vaccines developed from cultured mammalian cells, since plant viruses cannot infect humans. Edible vaccines act by stimulating the mucosal as well as systemic immunity, as soon they meet the digestive tract lining. This dual mechanism of action of edible vaccines provide first-line defense against pathogens attacking via mucosa, like *Mycobacterium tuberculosis* and carriers causing diarrhea, pneumonia, STDs, HIV etc. [2]. At present edible vaccines are produced for several human and animal diseases (measles, cholera, foot and mouth disease and hepatitis B, C and E). They can also be used to prevent exceptional diseases like dengue, hookworm, rabies, etc. by combining with other vaccination programs enabling multiple antigen delivery. Various foods under investigation for use in edible vaccines include banana, potato, tomato, lettuce, rice, etc. [3].

The role of a conventional vaccine

Vaccination is widely considered one of the greatest medical achievements of modern civilization. Childhood diseases that were commonplace less than a generation ago are now increasingly rare because of vaccines. Vaccines are designed to elicit an immune response without causing or spreading diseases. These are formed by killing or alleviating disease causing organisms. Hence, vaccines stimulate an immune response by the body that will fight off the respected type of illness it has been administered for. Widespread use of vaccines has reduced morbidity and mortality and extended the lifespan significantly. What were once common, life-threatening infections are now vaccine preventable diseases [4]. The traditional administration of vaccines via needle and syringe poses safety risks for patients, healthcare providers, and the community. A primary safety concern is the risk of transmission of infectious diseases between patients or between patients and healthcare providers [5, 6]. Vaccine development for many pathogens is presently constrained by the limited immune responses to vaccine antigens. This has caused novel approaches to rise.

The role of an edible vaccine

Edible plant vaccines are immunogenic preparations containing antigenic proteins rather than pathogens. Hence, they are able remove the possibilities of recurrence of disease by antigenic preparations containing organisms in any form. Expression of antigens as vaccines and of antibodies against antigens of pathogens in transgenic plants is a convenient and inexpensive source for various bacterial, viral, helminths, protozoan and autoimmune diseases with lower capital costs [7]. Edible vaccines are similar to subunit preparations, in which only the antigen is carried and bear no genes that would further enable whole pathogen to form. Therefore, edible vaccines are subunit vaccines where the selected genes are initially introduced into the plants and the transgenic plant is then induced to manufacture the encoded protein [8].

Mechanism of edible vaccine

Edible vaccines contain DNA fragments from the original pathogen. These fragments code for a protein that is usually a surface protein of the pathogen. This is responsible for eliciting the body's immune response [9]. Most pathogens enter at the mucosal surface lining the digestive, respiratory and urino-reproductive tracts, which are collectively the largest immunologically active tissue in body. The Mucosal Immune System (MIS) is the first line defense and the most effective site for vaccination against pathogens [10]. Hence, the success of an edible vaccine requires induction of the mucosal immune system (MIS). Induction of a mucosal immune response starts with the recognition of an antigen by specialized cells called M-cells. These cells are localized in the mucosal membranes of lymphoid tissues such as Peyer's patches within the small intestines. The M-cells channel the antigen to underlying tissues where antigen-presenting cells internalize and process the antigen. The resulting antigenic epitopes are presented on the APC surface, and with the assistance of helper T cells activate B cells. The activated B cells migrate to the mesenteric lymph nodes where they mature into plasma cells and migrate to mucosal membranes to secrete immunoglobulin (Ig) A. Upon passing through the mucosal epithelial layer towards the lumen, the IgA molecules complex with membrane-bound secretary components to form secretary IgA (sIgA). Transported into the lumen, the sIgA interacts with specific antigenic epitopes and neutralize the invading pathogen [11]. Orally administered vaccines are particularly efficient at stimulating local mucosal immune responses at the intestinal surface, and the integrated nature of the mucosal immune system allows other mucosal sites to also be primed [12].

Formation of edible vaccines

Gene encoding antigen from pathogenic organisms such as virus, bacteria, parasites that have been characterized and which have available antibodies are handled under two methods. The first methods includes the entire structural gene inserted into a plant transformation vector between regulatory elements 5' and 3', which will allow the transcription and accumulation of coding sequence in plant. In the second method, an epitope within the antigen is identified and DNA fragment encoding is used to construct genes by fusion with a coat protein gene from plant virus such as TMV or CMV. The recombinant virus is then used to infect the stabilized plants and the candidate vaccine antigens in plant tissues will be produced [13]. Edible vaccines are produced by introducing selected desired genes into plants and inducing the plants to produce the altered protein. Therefore, basically plant-based vaccine production mainly involves the integration of transgene into the plant cells. The target sequence of the selected antigen is integrated with the vector before being transferred into the expression system. The transgene can then be expressed in the plants either through a stable transformation system or through transient transformation system, depending on the location where the transgene has been inserted in the cells. Stable transformation system can be achieved through nuclear or plastid integration [14]. It is called stable or permanent due to the permanent changes occurring in recipient cells' genetics as the target transgene is integrated into the genome of host plant cells [15]. This is done by several techniques such as; agrobacterium gene transfer, biolistic method and electroporation.

i) Agrobacterium gene transfer method

Plant-based vaccine technologies involve the integration of the desired genes encoding the antigen protein for specific disease into the genome of plant tissues by various methods. Agrobacterium-mediated gene transfer and transformation via genetically modified plant virus are the common methods that have been used to produce effective vaccines [16]. Agrobacterium is a Gram-negative soil pathogenic bacterium that naturally will infect the plants and transfer their genes (T-DNA) to the nucleus of the plant cells [17, 18]. *A. tumefaciens* is the most preferred strain for stable expression of the desired protein as *A. tumefaciens* carries tumour-inducing plasmid (Ti-plasmid). There are genes in the Ti-plasmid that are responsible for encoding plant hormones such as auxin and cytokinin synthesis, which will induce tumour tissue in plants. In vaccine production, these genes will be erased to form neutralized Ti-plasmid and heterologous gene is inserted forming a recombinant plasmid vector [19]. The recombinant plasmid vector is transformed into *A. tumefaciens* and with the help from *vir* gene of the bacterium, the introduced heterologous gene is transferred by the transformed bacterium and integrated into the nuclear genomic DNA of the host plant by non-homologous recombination at random sites [17, 18, 19]. The drawbacks of this method are resultant low yield and slow process. Agrobacterium gene transfer method works best with dicotelydenous plants such as tomato, potato, tobacco [20, 21].

ii) Biolistic Method

Biolistic method is used as a method of transformation of plants, including monocotelydenous plants. The gene containing DNA coated metal (gold, tungsten) particles are fired at the plant cells using a gene gun [22]. Plant cells that can take up the DNA are allowed to grow in new plants and are clones to produce large number of genetically identical crop. This method is quite attractive since the DNA can be delivered into cells of plants which makes the gene transfer independent. Biolistic method can be used to achieve two types of antigen expression in the transgenic plants: nuclear and chloroplast transformation. Nuclear transformation is done by integrating the desired gene into the nucleus of the plant cells via non homologous recombination [23, 24].

iii) Electroporation

In this method DNA is inserted into the cells after which they are exposed to high voltage electrical pulse which is believed to produce transient pores within the plasma lemma. This approach requires the additional effort of weakening the cell wall as it acts as an effective barrier against entry of DNA into cell cytoplasm hence, it requires mild enzymatic treatment [8].

Advantages of edible vaccines

Edible vaccines have efficient mode of action for immunization, as they do not require subsidiary elements to stimulate immune response. Unlike traditional vaccines, edible vaccines are capable of bringing mucosal immunity. They are comparatively cost effective, as they do not require cold chain storage like traditional vaccines [25]. Edible vaccines contributes to higher storage opportunities as they seeds of transgenic plants contain lesser moisture content and can be easily dried. In addition, plants with oil or their aqueous extracts possess more storage opportunities [26]. Edible vaccines do not need sophisticated equipment and machines as they could be easily grown on rich soils and the method is economical compared to cell culture grown in fermenters and are widely accepted as they are orally administered unlike traditional vaccines that are injectable. Thus, they eliminate the requirement of trained medical personnel and the risk of contamination is reduced as they do not need premises and manufacturing area to be sterilized [27]. Edible vaccines promote opportunity for second-generation vaccines by integrating numerous antigens, which approach M cells simultaneously. Edible vaccines are safe as they do not contain heat-killed pathogens, hence do not present any risk of proteins to reform into infectious organism. Edible vaccine production process can be scaled up rapidly by breeding [8].

Disadvantages of edible vaccines

There is a probability for the individuals to develop immune tolerance to the particular vaccine protein or peptide. Dosage required for the edible vaccine preparation varies due to several factors such as the generation of the plant, plant itself, protein content, ripeness of the fruit and quantity of the food eaten [8]. Edible vaccine administration requires methods for standardization of plant material/product as low doses may produce lesser number of antibodies and high doses are responsible immune tolerance. These are dependent on plant stability as certain foods cannot be eaten in raw form and needs cooking that cause denaturation or weaken the protein present in it [28]. Since edible vaccines are plant based vaccines, they are prone to get microbial infestation. Edible vaccine function can be vulnerable due to vast differences in the glycosylation pattern of plants and humans [8].

Crops holding edible vaccines

Edible vaccines are formed in plant derived food. There are several crops used to create edible vaccines which are also known as transgenic plants. Mainly potatoes, tomatoes, lettuce and bananas are used as edible vaccine carrying crops. Other than that carrot, tobacco, rice, soy bean, wheat etc. are also used. These act as bio reactors to form subunit vaccines which are edible vaccines. Clinical trials have been conducted to experiment the effectiveness of the edible vaccines. The transmembrane glycoprotein (G) of rabies virus has been considered as the main antigen that induces neutralizing antibodies and many recombinant vaccines are based on this antigen to confer protection against lethal rabies infection [29], [30]. Successful expression of antigens in plants has been achieved for Rabies virus G-protein in tomato and tomato plants expressing rabies antigens has been developed to induce anti body production in mice [31]. Norwalk virus capsid protein in tobacco and potato [32], Hepatitis B virus surface antigen in tobacco and potato [33], E.coli heat-labile enterotoxin B subunit (LT-B) in tobacco & potato [34], Cholera toxin B subunit (CT-B) in potato has been developed under previous research studies [35].

Case studies for edible vaccines

Immunogenicity in humans of an edible vaccine for hepatitis B has been carried out as a case study. A double blind placebo controlled clinical trial has been used to evaluate the immunogenicity of hepatitis B surface antigen (HBsAg) which has been expressed in potatoes and orally ingested to previously vaccinated individuals. The levels of blood serum antibody titers against HBsAg have been used to evaluate the protection for hepatitis B. Uncooked potatoes have been given in 3 doses to 16 volunteers and 10 have showed an increment of 62.5% in serum anti-HBsAg titers. Transgenic potatoes have been given in 2 doses to 17 volunteers and 9 showed an increment of 52.9% in serum anti-HBsAg titers. Non transgenic potatoes were given to another set of volunteers and no change has been observed [36]. Transgenic tomatoes expressing human beta-amyloid for use as a vaccine against Alzheimer's disease has also been studied in detail [37]. Human β-amyloid (Aβ) is one of the main components of Alzheimer's disease. Reduction of Aβ is considered a key therapeutic target. Using *Agrobacterium*-mediated nuclear transformation, transgenic tomatoes have been generated for Aβ with tandem repeats. Integration of the human Aβ gene into the tomato genome and its transcription were detected by PCR and Northern blot, respectively. Expression of the Aβ protein was confirmed by western blot and ELISA. The transgenic tomato line expressing the highest protein level was selected for vaccination. Mice immunized orally with total soluble extracts from the transgenic tomato plants elicited an immune response after receiving a booster. The results indicate that tomato plants may provide a useful system for the production of human Aβ antigen [37].

Conclusion

Edible vaccines are produced by introducing selected desired genes into plants and inducing the plants to produce the altered protein. Plant derived edible vaccines may lead to a future of safer and more effective immunization. It is cost effective, stable under many conditions and it is a novel approach to oral immunization.

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