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A REVIEW ON MODES OF VACCINE DELIVERY SYSTEMS IN PHARMACEUTICAL INDUSTRIES

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ABSTRACT
Vaccines are developed to prevent the diseases by stimulating the immune response to act against the antigen. Many pandemic diseases can be prevented through vaccination. Vaccination can be done through different modes like parenteral, oral and intranasal. Vaccine technology is developing day to day to increase the efficiency of vaccination and to develop safe vaccines. Vaccines can be delivered through vehicles that can carry the antigen. Vaccine delivery vehicles play an important role in administering vaccines because certain vehicles are suitable and effective for certain types of antigens. Nanoparticles, organic compounds, polymeric materials can be used as vaccine delivery systems and can act also adjuvants. Plant-based oral vaccines or edible vaccines attracts scientists as it is cost-effective and can be easily administered. Many factors will influence the vaccine delivery. The environment where the vaccine is delivered should stabilize antigens and the antigens should release for immune response. Finally, vaccination is important and the mode of delivery will determine the effectiveness of vaccination.

KEYWORDS: Mode of vaccination, oral vaccination, edible vaccines, vaccine delivery vehicles.

1. INTRODUCTION

Vaccines based on live-attenuated or inactivated (killed) pathogens and inactivated toxins were developed in the golden age of vaccine when the germ theory was established by Pasteur, Koch, etc. The development of cell culture techniques leads to the second golden age of vaccines. The advancement of knowledge leads to new vaccines like subunit vaccines, polysaccharide vaccines, conjugate vaccines, synthetic vaccines, combination vaccines, recombinant DNA vaccines, reverse vaccinology, etc.[1] Vaccines were given to people in endemic regions through organizing campaigns. Mass vaccination camp has several factors to be considered. Some of the factors to be required are the temperature for storage and shelf-life of vaccines, the volume of clean water for the administration of the vaccine. In organizing camps for administrating Oral Cholera Vaccine (OCV) in endemic regions there were several challenges like planning to provide vaccines to target people in a stipulated time, waste disposal and environmental management and so on.[2] Multiple strains will be responsible for causing diseases like polio. So the vaccine preparation should include all the strains causing disease. Divalent, trivalent, tetravalent and pentavalent vaccines should be prepared in some cases. Sometimes a combination of strains can cause some other outbreaks because the presence or reaction takes place in the GI tract of any particular strain. The cessation of the trivalent oral polio (tOPV) vaccine was due to some outbreak caused by the second strain. Then the bivalent oral polio vaccine (OPV) was prepared and vaccinated then.[3]

Antigens can be incorporated in different modes such as parenteral mode through injection, oral administration and intranasal administration of vaccines. Intramuscular, subcutaneous and intradermal injections are modes of injecting vaccines through the parenteral method.[4, 5, 6,] Rotavirus vaccines are administered orally.[7] Nowadays, plants can be utilized to produce edible vaccines. In plant-based oral vaccination, the antigen is encapsulated in the plant cell. When the plants are consumed, the digestive enzymes break the cell wall and release antigens which increase the immune responses. Transformation technologies made possible to produce transgenic plants that can be incorporated with antigens. Agrobacterium mediated transformation can be done with particle bombardment.[8] Adjuvanted influenza vaccine is administered through the intranasal mode of vaccination.[9] Some microorganisms like Staphylococcus aureus are highly pathogenic and the vaccine development has so many challenges.[10] So the

vaccine delivery system plays an important role in increasing the efficiency of the vaccine. Several factors will be a limitation for proper immune response. Those factors influencing the immune response of vaccines need to be considered.

2. Parenteral mode of vaccination

Parenteral mode of vaccination is injecting vaccines into the skin. Needles are used to inject vaccines into the skin which may cause pain. Intramuscular, Subcutaneous and intradermal are the routes for parenteral mode of vaccination. In intramuscular route, vaccine is delivered in the muscular layer of the skin. Intramuscular (IM) vaccination with a heat-inactivated Mycobacterium bovis vaccine (IV) was administered to control tuberculosis (TB) in the wild boar.[11] Parentally administered, a non-replicating rotavirus vaccine showed a promising solution for vaccinating infants. The outer capsid protein of the rotavirus is administered intramuscularly to the infants which showed good immune responses and has some advantages over oral rotavirus vaccine.[12] Vaccine is delivered into the subcutaneous layer of the skin *(i.e)* a layer above the muscular layer in subcutaneous vaccination. Subcutaneous vaccination will be better way for antigen delivery in Nano disc form than intramuscular injection. This Nano disc subcutaneous injection can be used to stimulate T cell immune response in cancer immunotherapy.[13] Vaccine is delivered to the top layer of the skin in intradermal injection. Little volume of vaccine can be injected through intradermal mode. Intradermal (ID) adapter, Star ID syringe, disposable syringe jet injector tropis and micron jet 600 are some of the devices for injecting intradermal vaccines. Fractional dose of inactivated poliovirus vaccine (fIPV) can be administered intradermally. Some more additional research is needed to implement this fIPV administration.[14] Intradermal vaccination of zostar vaccine could increase immunogenicity when compared with subcutaneous mode of injection.[15]

2. Oral vaccination

Vaccine is delivered orally to create local protection against pathogens. As the expensive reactors and expensive methods are used to produce protein drugs, the cost of protein drugs are very high .Oral vaccine can be produced in low cost. It also has the advantage that proteins can be easily digestible by the gastric enzymes and utilized by the body to control diseases.[16] In plant based oral vaccination, the antigen is encapsulated in the plant cell. When the plants are consumed, the digestive enzymes break the cell wall and release antigens which increase the immune responses. Transformation technologies made possible to produce transgenic plants which can be incorporated with antigens. Agrobacterium mediated transformation can be done with particle bombardment.[17]

2.1. Plant based vaccines

Agro bacterial transformation made a vast development in producing transgenic plants. The ability of transferring the bacterial genome into plant genome laid the foundation to produce our targeted protein or toxin in plants. Transgenic plant development attracts scientists to develop plant based oral vaccines. Transgenic potato marker-free plants were transformed to carry hepatitis B surface antigen (HBsAg). Enzyme linked immunoassay (ELISA) of the serum of immunized animals showed an increased level of HBsAg antibodies and booster vaccination showed the immunological memory after the intraperitoneal injection. Transgenic potato will be a promising substance to produce edible vaccines against hepatitis.[18] The pathogens like hepatitis C virus(HCV) which has high genetic diversity can be produced in plants through transient expression of genes to the nucleus. HCV E1E2 heterodimer polypeptide and E1E2ΔN6 were expressed in lettuce (*Lactuca sativa*) through Agro medium-mediated transient expression. Compared to intramuscular injection, oral feeding showed good immune response.[19]

Some non-protein drugs which are effective against the specified pathogens and expensive to process the drug are produced in plants artificially with low cost. The metabolic processes to produce the drugs are engineered at different cellular compartments. Artemisinin, a non-protein drug effective against drug resistant malarial parasites is engineered to produce in tobacco plants.[20] Heat-labile enterotoxin B (LTB) and cholera toxin B (CTB) subunit was expressed in rice *(Oryza sativa*) to prevent diarrhea. There is an increase in levels of antibodies against the enterotoxigenic strains of *E. coli* and *Vibrio cholera*.[21]

Not only toxins but also the viral capsid proteins can be expressed in transgenic tobacco and potato.[22] Hairy root of tomato was chosen to produce engineered fusion protein of rabies glycoproteins and ricin toxin B chain antigen (RGP-RTB).[23] Carrot cells are used as vehicles in which recombinant β-glucocerebrosidase (prGCD) was expressed. As the GCD was stable in gastric environment, it is suitable to use as vehicle. The stability in the gastric environment was found by immunoblot analysis. The uptake of prGCD was studied and the concentration of prGCD is increased in the small intestine as the plasma cells absorbed prGCD.[24]

The human or animal virus epitopes can be expressed in plant viruses which then be transferred to plant. The epitope mapping helps to find short amino acid sequence which is highly immunogenic. This helps to express immunogenic epitopes in plant genomes[25] and also the method for producing plant based oral vaccines are cost effective and promising solution for effective vaccination.[26]

3. Intranasal vaccination

Vaccine is delivered through nasal route. Epithelial layer of nasal area has M cells (microfold –cells) which are present in the over layer of nasal-associated lymphoid tissue(NALT). These cells in nasal route has the capacity to react for antigen and the mucosal region produce immune response. This property is helpful to administer vaccine through nasal route.[27] Nanoparticle vaccine delivery attracts researchers to deliver intranasal vaccine. Biodegradable particles can be used as delivering systems. Chitosan nanoparticles based intranasal vaccine delivery is a promising system to make the vaccination effective. The size of delivering vehicle, biodegradability, biocompatibility of chitosan favours the use in vaccine delivery. Glycol chitosan nanoparticles show greater immune response than chitosan nanoparticles. Zeta potential value and better mucoadhesive ability determines the effectiveness of vaccine delivering system through nasal route. Hepatitis antigen carried by glycol nanoparticles showed better immune response than the antigen carried by chitosan nanoparticles.[28]

4. Vaccine delivery vehicles

Vaccines are delivered through delivery vehicles which will be encapsulated with the antigens. Vaccine delivery vehicles should be target specific and should act as adjuvant in some kinds of antigen. Concerning oral vaccines, it should stabilize in the varying pH of gastrointestinal tract (GI).The stability of vaccine in GI tract and the immunogenic activity in GI tract of the vaccine delivery vehicle should be tested before commercialisation of vaccines. Drugs which are not soluble in water and degraded in gastric environment are delivered to humans through nanoparticles which are widely developed as oral vaccine delivery systems. Layer by Layer (LBL) milk protein casein (CN) coated with iron oxide nanoparticles is used to deliver doxorubicin (DOX) drug which is incorporated into inner polymeric layer. The positive results of the stability of nanoparticles which are the release of drug and the uptake of drugs by the epithelial layer shows promising oral drug delivery Nano platform.[29] Similarly chitosan nanoparticles are used to entrap antigens which are used as vaccine delivery system. During the preparation of polymeric nanoparticles pH sensitive polymer is used to withstand the gastric pH. Ionic gelatin technique was used to fabricate Trimethyl chitosan (TMC) nanoparticles and hydroxypropyl methylcellulose phthalate (HPMPC) is used as pH sensitive polymer in some works.[30] Guar gum nanoparticles can be used as oral vaccine carrier for tuberculosis.

Not only nanoparticles inorganic compounds can also be used as oral vaccine delivery vehicles. Carbon materials are used as delivery vehicles. The S-3DC material has high protein adsorption capacity owing to macro porous structure. It has special internal structure which made it a repository-type carrier.[31]

Viruses which are surviving in mucosal regions and their ability to cross the epithelial layer cause infection. Some critical infection like HIV cause serious problem.p24HIV antigen can be orally delivered to control infection in mucosal regions. Salivary antigen specific (SIgA) can be used as vaccine carrier for an HIV antigen which targets immune response in gastrointestinal environment.[32] Virus particles like alphavirus replicon particles are used to carry expressing antigens. Fluorescence Microsphere Immune Assay (FMAI) is a method to detect multiple targets at the same time.[33] Alphavirus replicon nanoparticles were also used as adjuvants in oral vaccination against ISAV in salmon. Adjuvants normally used to increase the efficiency of the vaccine and also as supporting agent.[34]

There are some site specific oral delivery vehicles like T-HPMCP (thiolated-hydroxypropyl methylcellulose phthalate). Attuning hydroxypropyl methylcellulose phthalate is used for selective delivery of protein vaccine in ileum.It target M cells in ileum. To avoid release of antigen or protein in the parts other than ileum T-HPMCP was used.[35] Thiolated eudragid (TE) paricles can also be used as antigen carrier which targets M cells.[36] Antigens can be encapsulated in poly lactide-co-glycolide (PLGA) polymers. When the immobilized antigen was delivered orally through PLGA microspheres in fishes to protect against rainbow trout,there was observed good relative percentage survival(RPS) values of groups. There is an increased RPS value when booster immunization was done after the intraperitoneal injection.Booster application of SA and PLGA particles lead to a efficient level of immunization. So vaccine carriers can be polymers too.[37]

DNA vaccines can be prepared to produce vaccines. The proteins which are difficult to prepare a soluble recombinant protein, DNA vaccine preparation shows an alternative approach.[38]

5. Factors influencing effective vaccine delivery

Various studies need to be carrying out before producing oral vaccines. Some of the studies include morphology, particle size, particle distribution, antigen loading efficiency, acid degradation protein assay, *invitro* release studies, payer's patch uptake study, invivo studies like specific antibody release study etc.[39] In each study there are several factors needed to be considered to prepare oral vaccine. The dosage of vaccine will affect the immune response. The high antigen dose (HiAg) showed high antibody production compared to low antigen dose (LoAg) in oral vaccination of rohu using PLGA nanoparticles coated with outer membrane protein (OmpW) of *A. hydrophila*.[40]

When hydrogel degradation is used to deliver protein orally, several factors like protein size and crosslinking density should be considered. For small peptides like salmon calcitonin, high concentration (5% and 10%)

formulation of crosslinks showed best results of releasing protein. For large antibody like rituximab, low concentration (1%) formulation of crosslinks showed better results. While the 5% and 10% cross-linked formulations proved best for the small peptide salmon calcitonin, the 1% cross-linked formulation was vastly better for the large antibody rituximab. The concentration of crosslinks determines the pore size for diffusion of proteins through hydrogels. The protein size plays as an important factor for oral delivery of proteins.[41]

The appropriate validation of the gene array data will provide better information the function of gene.The gene which will be encoded should be express in right manner. Some transcription factors may alter the expressing system or affect the normal gene expression. Many pathways will affect the gene expression. So, all the factors should be considered before preparing vaccines.[42] Stress also alters the immune response produced by the body. This stress factor is considered based on the study done for immune responses against influenza virus.[43]

The antigen loading methods also matters for immune response. Either encapsulation or physical adsorption of antigen, the process of loading this antigen to vaccine carriers like PLGA is to be considered. Adjuvants used to enhance vaccination should be a right material which should have proper function.[44] Sex also influences vaccination because both male and female has different pattern of immune system. Immune responses will be different for both male and females. Vaccine preparation should consider the sex too and then only vaccine can be delivered.[45] Microarray provides better platform to study the antibody responses. Novel antibody targets can be identifying through microarray. Effective vaccination can be achieved if microarray was used.[46]

CONCLUSION

As vaccines have more benefits, vaccine drug delivery systems gaining popularity in this scientific community. The advancements in genetic engineering promote vaccine delivery method. Edible vaccines are an attractive way for oral delivery of vaccines. Some surface modifications of carriers will enhance the vaccination. Needle free vaccination has some advantages like no pain transferring of disease through needle over other modes of vaccination. Vaccination modes and delivering systems mainly influence the efficiency of vaccination.

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