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NANOEMULSIONS AS COADYUVANTS IN INTRANASAL VACCINES

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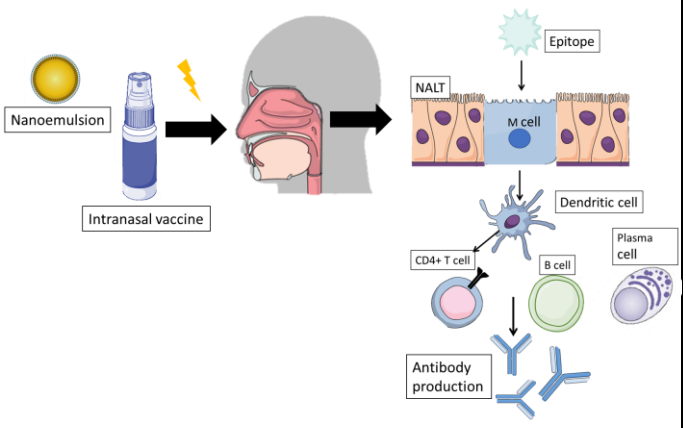
Graphical Abstract



Abstract.

A considerable amount of human infections take place in mucous of the body, which is the reason why it is important to trigger immunological protection in this area. Even though parenteral administration of antigen induces efficient systemic protection, mucous from the body keep unprotected. Although attempts have been made to activate immunological protection in mucous membranes through different routes of application, some of them have had side effects or the desired effect has not been achieved clinically. [1]

Nasal mucosa route has been studied for drug administration and for vaccines application in recent years using peptides/proteins or genetic structure materials. Among the advantages reported on this material, due to the nature of these ingredients, it is susceptible to enzymatic degradation processes, so its half-life is very short. In addition, they are not able to easily cross the mucous membranes of the body or the biological membranes. [2] This led to the research and development of nanocarriers as coadyuvants, to protect these materials and give them more stability.

Research shows that mucosal vaccination has advantages over intramuscular immunization when it comes to provides mucosal protection, probably the most important of them is the fact that intranasal administration induces humoral and cellular immunity, which allows immunization at different mucosal sites as well as nasal mucosa, as well as systemic protection. [3]

Introduction

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NASAL ROUTE: ADVANTAGES AND DISADVANTAGES

Intranasal administration route is a non-invasive route, especially for respiratory diseases. [3] This route allows the use of heat-resistant vaccines and does not require needles for their application, so they can potentially reduce costs of their use since no special personnel is required for their administration or waste disposal, which would favor mass vaccination campaigns. In addition to that, the intranasal route allows the administration of both liquid and dry vaccines. [2] Preclinical studies have showed that intranasal dry powder vaccines (DPVs) is a convenient way to trigger immunological protection and they are more stable than liquid vaccines. [5]

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It has been demonstrated in animal models that nasal vaccination generates protection against antigens from influenza virus, recombinant anthrax protective antigen, HIV gp120, and hepatitis B surface antigen (HBsAg). [4]

As compared to the nasal route, the pulmonary route offers a much larger mucosal surface area and has a great vascularization in the alveolar tissue. This may facilitate the systemic delivery of antigens and increase respiratory and systemic immunization. The dry powder vaccines (DPVs) by inhalation has already been studied for some diseases like tuberculosis, hepatitis, influenza, and measles. [12]

Due to particles above 5 micrometers get trapped on the surface of the upper airways, it is necessary for particles to reach the deep lung measure between 1 and 5 micrometers. So, the formulation and the device used to administer the formulation are critical, because they determine the particle size for lung deposition. However, for vaccine delivery, it may be not totally a problem because the administrated antigens can still reach the lymphoid tissue in the oropharyngeal region. [12]

IMMUNOLOGICAL RESPONSES TRIGGERED BY ANTIGEN NASAL ADMINISTRATION.

The mucosal response has been attributed to the internalization of the nanoemulsion droplets and activation of Toll-Like-Receptors (TLR), specially TLR-2 and TLR-4. [3] Nasal administrated antigens

can follow up two routes: soluble antigens are able to potentially penetrate nasal mucosa and activate antigen-presenting cells (APCs), like dendritic cells and macrophages, however, particle-sized antigens may either be eliminated by the mucociliar system or may be absorbed by microfold cells (M cells) in NALT. [5]

If APCs are activated, they trigger the immune response of T and B cells, so they differentiate in plasma cells and release immunoglobulin A (IgA) in the lumen, which neutralizes specific antigens and forms complex antigen-antibodies, so they get trapped easily in the mucus and later ciliary movement of the epithelial cells eliminates them. Lymphocytes that were activated in a specific mucosal surface, can travel through the lymphatic system and reach remote sites of the mucosa, thus providing protection also in this new site. This process is called "the immune system of the common mucosa." [5]

Antigens captured by NALT drain into the lymph nodes, thus generating the production of serum IgA and IgG in the systemic lymphoid organs. However, the systemic immunological protection caused by mucosal vaccination is usually less effective than that induced by parental immunization. [5]

The ciliary clearance might be a problem to nasal vaccines, because antigens would not be able to trigger immune response adequately before they are eliminated, however, chitosan has been already proved as a mucoadhesive to prolongate the time antigens are in contact with immune effector sites and descendental immunological response. [5]

NANOEMULSIONS AS ADJUVANTS HELP WITH DISADVANTAGES OF THE NASAL ROUTE

In the last years, nanoemulsions (NEs) have been studied as adjuvants in nasal mucosal vaccinations due to their immunostimulatory and/or immunomodulatory effect. NEs provide antigens more stability because the antigen gets entrenched into the oil droplets, preventing degradation of epitopes. [3]

Emulsions are dispersions of two immiscible liquids, usually oil in water (O/W), which contain 5–20% lipid [6], nonetheless they can also be water in oil dispersions (W/O), stabilized through surfactants. Depending on the particle size and stabilization, emulsions are categorized in macroemulsions, nanoemulsions and microemulsions. [3] Particularly, nanoemulsions (NEs) are transparent, translucent, and non-stable colloidal dispersion phase droplets in the nanometric scale, about 100nm, but some bibliography also refers limit up to 300nm [3,5]. They can also be referred as ultrafine emulsions, submicron emulsions, translucent emulsions and miniemulsions. [7]

Unlike microemulsions, NEs cannot be formed spontaneously, so it requires high energy methods to prepare them, usually by high pressure homogenizers, high-shear stirring and ultrasound generators. Proteins and other thermolabile compounds, such as enzymes and nucleic acids, may suffer deterioration during the process due the high pressure and temperatures. [7] However, there is another alternative to prepare NEs, which consists in low-energy methods such as phase inversion temperature and emulsion inversion. [6]

When it comes to nasal drug delivery, NEs are characterized by a higher surface area compared to other formulations, and they can be used to solve problems of drug solubility and/or of drug stability such as oxidation, pH, hydrolysis and enzymatic degradation at the mucosal level, under physiological conditions. [6]

Though, some resources show that even though NEs have good stability, their droplet size may follow the Ostwald ripening process and increase over time or may even breakdown. Solutions with a high solubility or highly polydisperse systems may suffer micellar based diffusion or follow a passive via which leads to a larger sized droplet at the expense of the smaller ones. [3]
Nanoemulsions only have kinetic stability and no thermodynamically [3,7] Having long-term physical stability due to the small size of drop prevents destabilization processes such as coalescence, flocculation, creaming, and sedimentation during storage time. [3, 6]

Emulsifiers give NEs stability against heating, cooling, pH, ionic strength and long-term storage. [8]. Surfactants provides stability as ionic surfactants provide electrical charge whereas non-ionic surfactants create a steric barrier with bulky molecular groups. Thit is the reason why the emulsifier used for NEs is usually a surfactant, but proteins and lipids have also been reported. [3, 6]

Some of the more used are lecithin (phosphatidylcholine), sodium deoxycholate (bile salt), polyoxyethlene sorbitan monolaurate, and sorbitan monolaurate. Others are poloxamers, sodium dodecyl sulfate, amphiphilic proteins like casein, polysaccharides (starch derivatives, gums) and poly-ethylene-glycol (PEG)-containing block copolymers. [6]

The development of an intranasal vaccine requires nanocarriers capable to enter the mucosal cells and deliver antigens to the cells. Among the 5 types of nanoparticles studied, the biodegradable nanoparticles based on porous and cationic maltodextrin were the best option for the administration of proteins. [13]

Co-surfactants, such as polyethylene glycol, ethylene glycol, propylene glycol, ethanol and glycerine also can be used for the stabilization of NEs. These formulations seem safe because mutagenic effects have not been reported in literature. [6]

Makidon and collaborators proved if administrated antigens suffer functional changes due to protein unfolding and concluded that nanoemulsion based vaccines do not require engineered designed delivery devices to be effective. [3]

CURRENT APLICATIONS

FluMist Quadrivalent is a live attenuated influenza vaccine (LAIV) indicated for immunization against influenza A subtype viruses and type B viruses. [9]

Even though the American Academy of Pediatrics (AAP) and Centers for Disease Control and Prevention (CDC) did not recommend using LAIV in 2016, 2017 or 2018 due to poor effectiveness

against H1N1 strains, since it has been reformulated, in 2019 AAP announced it does not have a preference between LAIV and the inactivated influenza vaccine. [10]

The FluMist Quadrivalent 2019 version protects against 4 weakened influenza viruses: A (H1N1), A (H3N2), B Yamagata lineage, and B Victoria lineage. Its inactive ingredients are monosodium glutamate, gelatin, arginine, sucrose, dibasic potassium phosphate, monobasic potassium phosphate, and gentamicin. [9]

Probably the most important condition that may have a detrimental effect during its administration is in children under 5 old with recurrent wheezing and persons with asthma no matter the age, because after its administration they may experience wheezing. However, it has not been studied in persons with severe asthma or active wheezing. [9]

There have been already commercialized intranasal vaccines such as FluMist®, which is the first live attenuated influenza virus IN vaccine that was approved and commercialized in the US and Europe as Fluenz®. Nasovac® is an influenza vaccine approved in India. Other vaccines for IN administration have been studied against infectious diseases like measles, meningitis, tuberculosis, and pneumonia. [12]

Conclusions

Because of its many advantages, its wide use and its appropriate cost-benefit, it is intranasal route is proposed as a great option to vaccines administration, especially for mass vaccination to prevent epidemics.

Despite the challenges of its conservation and activity that have arisen, we encourage to continue investigating intranasal vaccination with the use and testing of nanoemulsions as adjuvants, which induce and increase immunological activity, in addition to providing stability to the components of the vaccine. This in order to have new options to use intranasal vaccines in the future against a greater variety of diseases, providing immunological protection on mucous membranes and systemically against antigens, with the least possible number of contraindications.

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