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Live attenuated and inactivated viral vaccine formulation and nasal delivery: Potential and challenges☆

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ABSTRACT

Vaccines are cost-effective for the prevention of infectious diseases and have significantly reduced mortality and morbidity. Novel approaches are needed to develop safe and effective vaccines against disease. Major challenges in vaccine development include stability in a suitable dosage form and effective modes of delivery. Many live attenuated vaccines are capable of eliciting both humoral and cell mediated immune responses if physicochemically stable in an appropriate delivery vehicle. Knowing primary stresses that impart instability provides a general rationale for formulation development and mode of delivery. Since most pathogens enter the body through the mucosal route, live-attenuated vaccines have the advantage of mimicking natural immunization via noninvasive delivery. This presentation will examine aspects of formulation design, types of robust dosage forms to consider, effective routes of delivery (invasive and noninvasive), and distinctions between live attenuated or inactivated vaccines.

Contents

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1. Introduction

Vaccines are antigenic substances that elicit an immune response, providing protective immunity against a specific or closely related pathogen. A hallmark of an effective vaccine is a long-term protection to the immunized individual from the pathogen upon re-exposure to that agent at a later time. The immune system responds in the generation of neutralizing antibodies (humoral response) against a specific pathogen. Vaccines may be of bacterial or viral in origin. Few live attenuated bacterial vaccines have become commercially available primarily because of safety and poor stability issues in such cases [1]. A vaccine contains an agent resembling a disease-causing microorganism with attenuated virulence and potent antigenic properties. Some vaccines are made from live-attenuated, killed, fractions or substances produced by the same pathogenic organism. This work will primarily focus on viral vaccines and emphasize the live attenuated and inactivated viral vaccine types. Nevertheless, much of the information discussed pertaining to viral vaccines may be applied to live and inactivated bacterial vaccines recognizing that compositional differences need to be taken into account.

Table 1 shows an assortment of the commercially available live attenuated vaccines currently on the market. Some general observations from the table are that most listed are formulated as dry solids with influenza and rotavirus being the exceptions as liquid dosage forms. There are formulated cases (Rotarix, Vivotif, and RotaTeQ) that can be delivered orally. Polio vaccine is a historic example of another live attenuated dosage form that can be administered orally but the disease has since been eradicated within the United States. Among the orally administered dosage forms, two are solid (Rotarix and Vivotif) and two are liquid (RotaTeQ, Rotarix). Only FluMist™ is administered intranasally using an Accuspray device, the rest by scarification, intravesicular or subcutaneous routes. Dose volume ranges are also listed in the table and range from 0.0025 mL (bifurcated vaccination needle; ACAM2000) to 53 mL (intravesicular; TheraCys) with corresponding dosing frequency.

Table 2 shows a listing of commercially available killed (inactivated) vaccines. Approximately 16% are solid and 84% are liquid dosage forms. Most of the liquid dosage forms are inactivated influenza viral vaccines, while the solid dosage forms involve a variety of immunizations against other diseases (e.g., rabies, meningitis, diphtheria, tetanus, poliovirus, and pertussis). Most are administered intramuscularly with few administered via subcutaneous or intradermal routes. Dose volumes range from 0.1 mL (Fluzone-ID) to 1 mL.

1.1. Live-attenuated vaccines

Live-attenuated vaccines generate strong humoral as well as cell-mediated immune responses (CMI). Disease-producing ("wild") viruses or bacteria are manipulated *in vitro* to reduce pathogenicity. Some attenuation in pathogenicity can occur through genetic alterations leading to the absence of toxins that make up the vaccine [2]. A small dose of the attenuated pathogen is required to replicate in an inoculated individual, capable of triggering an immune response with minimum virulence. They are usually administered by the natural route of infection. Moreover, the attenuated pathogens induce lengthy immune responses without adjuvants (an immunological agent that boosts immunogenicity) and are able to survive the low pH, enzymatic environment, of the stomach. In theory, these types of vaccines are safe and devoid of side effects, although there have been vaccine-associated effects reported. For example, paralytic polio has been linked with an oral polio vaccine (now discontinued) and intussusception associated with a recalled rotovirus vaccine (RotaShield) [3,4]. There are practical challenges associated with some live-attenuated vaccines. For example, refrigerated storage and distribution (i.e. "cold chain") requirements for liquid formulations are a significant hurdle in third world countries.

Fig. 1 illustrates the differences between the component parts of different virus types and their respective compositions. From the figure it can be seen that there are enveloped and non-enveloped viruses. The enveloped types have lipid bilayer membranes with an assortment of protein receptors transmembraneously attached to the surface. The lipid bilayer houses the capsid protein (and other proteins) and genome materials (Fig. 1A). In contrast, the non-enveloped variety lack the lipid membrane but have capsid and other proteins that house the genome material and may have a variety of different protein compositions with receptors for docking and fusing with host cells. Thus for enveloped viral vaccines, the general composition includes lipid structure, proteins, and genome (either DNA or RNA) in contrast to proteins and genome for non-enveloped vaccines (Fig. 1B). The type of viral vaccine and molecular compositions should be taken into account when considering vulnerability to a variety of stresses and instability.

Lipid bilayers can be composed of glyco-lipids, glyco-proteins, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine. They may also contain cholesterol and oleic acid. Enveloped viruses derive their assorted phospholipid and other compositions from the host cells they bud from [5,6]. In enveloped viruses the integrity of the lipid bilayer can be important for infection. Some evidence has been reported in the case of myxoviruses (influenza and paramyxoviruses) regarding the role of the membrane in fusion processes [7,8]. Furthermore, a high cholesterol to phospholipid molar ratio within viral envelopes has been noted as a requirement for infectivity [9]. Therefore, stresses that impact this membrane integrity can also impair the efficacy of the viral vaccine.

1.2. Killed-inactivated vaccines

Killed-inactivated vaccines are made from disease-causing microbes that have lost or attenuated their ability to infect through physical, chemical or radiation processes, without compromising the antigenicity of the microbial agent. The term killed commonly refers to bacterial vaccines, whereas inactivated relates to viral vaccines. These types of vaccines do not generally elicit a full cellular immune response [10]. Syringes are the most commonly used method of administration worldwide. To overcome weak immune responses, some inactivated vaccines (e.g., protein subunit varieties) are often co-administered with an adjuvant with large and more frequent doses than would be anticipated for live-attenuated vaccines. Moreover, adjuvants could cause local reactions at the vaccination site or contribute to other aspects of reactogenicity [11]. The major challenges of the inactivated vaccines are formulation stability, ability to generate long-term immunity and safety.

1.3. Immune response

Besides creating stable vaccine formulations, the delivered antigenic agents need to trigger the immune system to produce neutralizing antibodies particular to that disease. The immune system can be divided into innate and adaptive roles. The innate system (non-specific involving