AEROSOL VACCINES – PERSPECTIVES AND THERAPEUTIC IMPACT

Stanimira Zasheva[1], Viliana Gugleva[2], Velichka Andonova[2]

[1]Student, Faculty of Pharmacy, Medical University of Varna
[2]Department of Pharmaceutical Technologies, Faculty of Pharmacy, Medical University of Varna

ABSTRACT

INTRODUCTION: Aerosol vaccination is a promising non-injectable method that provides immune resistance to pathogens since it follows the natural route of transmission of many infectious agents. The immune response, occurring after intranasal or respiratory vaccine administration, provides credible protection due to the common mucosal immune system, excellent blood supply, and lung permeability.

AIM: The study aims to highlight the unconventional and perspective application use of aerosol dosage forms as a technological approach for vaccine drug delivery.

MATERIALS AND METHODS: A detailed literature survey in scientific databases such as PubMed, ScienceDirect, ResearchGate has been conducted, and the relevant information has been summarized and interpreted.

RESULTS: The aerosol vaccination method, as an alternative to the subcutaneous, intramuscular or intradermal application route, ensures defense against the inhaled pathogens, avoiding at the same time drawbacks associated with the injection administration such as the risk of reuse of disposable medical consumables, potential spreading of blood-borne diseases and the necessity of qualified medical personnel. Additionally, aerosol vaccination is an easier and more practical approach for patients, leading to improved compliance. Vaccines applied by the inhalation or nasal route of administration could be a successful approach for the treatment of diseases such as measles, tuberculosis, or influenza A, that although preventable, remain a global challenge.

CONCLUSION: The aerosol method is promising for vaccine delivery with the potential to be fully evaluated in the upcoming years.

Keywords: *influenza, inhalation, immunity, measles, nasal route, respiratory tract, tuberculosis*

Address for correspondence:
Viliana Gugleva
Faculty of Pharmacy
Medical University of Varna
84 Tzar Osvoboditel Blvd
9002 Varna
e-mail: viliana.gugleva@gmail.com

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INTRODUCTION

Regular immunization remains the primary approach for the control and prevention of various diseases. The most often applied vaccines are administered by injection (intramuscular, subcutaneous, or intradermal) directly into the bloodstream providing high bioavailability of the active ingredients (e.g., antigens). By the injection method, a precise

dose can be easily ensured as well as a systemic immune response (1). However, the application of subcutaneous, intramuscular, and intradermal vaccines faces some challenges and drawbacks. For instance, parental vaccines lead to temporary local immunity. Whereas the active ingredients are widespread in the blood circulation, their concentration at the portal of entry, usually the mucosa of the respiratory and gastrointestinal tracts, is quite low (2).

Additionally, developing countries have low access to vaccines due to the absence of medical personnel and insufficient vaccine import. Another limitation associated with injection includes a potential risk of reuse of disposable needles or syringes, hence possible transmission of local and systemic infections such as AIDS and hepatitis (3). The parenteral administration of vaccines is also associated with inconvenience and pain that lead to a lack of compliance especially by children (4). Last but not least, injections carry many risks for the people by incorrect storage or application, such as local reaction (swelling, bruising, inflammation, etc.) or toxic shock syndrome in case of non-sterile products or procedures (5).

A promising alternative compared to the parenteral vaccination appear to be the nasal and inhalation routes. The aerosol application could provide a rapid immunization, primarily in the epidemic areas, where time-saving is a critical issue (6). The respiratory and nasal vaccinations are proven to be safer and more immunogenic in lower doses than injections. The nose and mouth are easily accessible, do not require the use of needles or syringes; therefore, the risk of infections is minimal (7). Aerosol vaccines can be applied easily by patients themselves, without the necessity of a hospital environment or causing stress to the patients (4,6).

Furthermore, the inhalation and nasal routes are reported as suitable for the administration of several antigens at once (8,9). Aerosol vaccines show a better boosting response than subcutaneous ones. The outcomes from conducted studies indicate an increased resistance to virus exposure and more extended protection in infants of vaccinated mothers (10). The aerosol vaccination methods could be applied successfully in diseases such as measles, influenza A, anthrax, and tuberculosis, whose elimination is still a priority nowadays (8,11,12,13).

Although numerous aerosol vaccine formulations have been elaborated and studied in clinical trials, their practical application remains a challenge. Some of the limitations associated with their widespread use include factors such as high expenses for materials and equipment, which increase the economic price of aerosol vaccines in general, technical difficulties in the formulation process, and potential environmental issues (14). From a technological aspect, strict control regarding particle size must be imposed to ensure drug penetration through the epithelium (15). In this context, the administration of the vaccine in the form of micro- and nanometer-sized particles is characterized by increased efficiency and drug delivery (16). From biopharmaceutical point of view, factors such as the vital capacity of the lungs and airway condition should be considered since diseases like chronic obstructive pulmonary disease severely hinder the penetration of drugs administered via aerosols into the respiratory system; thus, leading to variations of dosages (17). With the need for consideration and optimization, aerosol vaccines are a promising and safe approach for mass immunization, an object of numerous clinical trials, whose potential is yet to be fully evaluated.

AIM

The study aims to summarize the relevant information about aerosol vaccines, to review their advantages and limitations, and to discuss their clinical effectiveness.

MATERIALS AND METHODS

Comprehensive research in the scientific databases PubMed, ScienceDirect, ResearchGate regarding characteristics and potential use of inhaled and intranasal applied vaccines in the last twenty years has been conducted.

Route of Immunity Introduction

The point of entry of respiratory antigens plays a significant role in immunity due to the mucosal immune system (18,19). The latter consists of epithelium and underlying connective tissue, impregnated with immune cells, nasal-associated lymphoid tissue, lymphoid tissue of the larynx and bronchi, and numerous lymph nodes, dispersed along the respirato-

ry tract (20). The epithelium contains four major immunocompetent cell types – macrophages, lymphocytes, dendritic, and M-cells (18,19). M- or MALT-cells (mucosa-associated lymphoid tissue) are considered as the main reason for the occurrence of strong local respiratory immunity (18).

Nasally applied vaccines mostly impact the local protection of the organism, as opposed to the inhalation route of administration, which is associated with systemic action. The epithelium of the respiratory tract is highly vascularized and covered in microvilli, which form a large absorption surface (5,16). Through the thin mucosa layer, especially in the alveoli, inhaled substances can penetrate the blood and lymph, where T-cells can contribute to a generalized immunity (20). Limitations associated with the inhalation route include its unsuitability for asthmatic patients due to possible inaccurate dosing since the penetration through the inflamed mucosa is not thoroughly studied, and dose administration additionally depends on the patient's inhalation capability (21,22). The use of nasally applied vaccines is not recommended to people with nerve disorders due to the single reported cases of facial paralysis associated with the application of intranasal influenza vaccine (17,18).

However, in terms of being used as an alternative to injectable vaccines, aerosol vaccines are considered suitable since according to conducted trials, they show better tolerance and fewer side effects (Fig. 1).

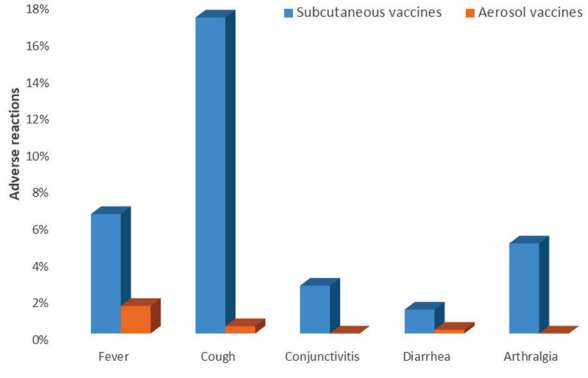

Fig. 1. Adverse reactions by route of vaccine administration (8)

Specifics of Aerosol Dosage Forms

Depending on the delivery path – nasal or by inhalation, aerosol particle sizes should be taken into consideration, especially in the case of nebulized solutions aiming to deliver small particles into the lower respiratory airways. According to the performed studies, ultrasound mesh nebulizers generating particles within 1 – 5 μm in diameter have been reported to be the most efficient for deposition to the lower lungs (Table 1) (24). For 30 seconds, about 0.1 mL of vaccine can be delivered by aerosol, which is equivalent to the antibody import provided by injection. Hence 5 mL are sufficient for the vaccination of 45 children (25). Nanoparticles are also reported to be able to reach the peripheral lungs and significantly increase the permeability and reactivity of the immune system to pathogens (18,24). Concerning the impact of size and size distribution for effective drug delivery, nasal sprays providing particles wider than 5 μm are suited mainly to reach the upper respiratory mucosa. They are usually deposited at the oropharynx due to ciliary action (18).

Table 1. Predicted aerosol deposition of different size particles in adult lungs (24)

Particle Size	Lung Deposition
>5 μm	Trapped in the upper airways
1-5 μm	Deep lung deposition
<1 μm	Diffusion through the pulmonary alveoli
500 nm	Phagocytosis by alveolar macrophages

Dry powders, suspensions, and solutions are reported to be used as delivery systems for aerosol vaccination (13,18,25). Initially, solutions for nebulization were studied as an aerosol vaccination alternative. Afterward, dry powders were investigated due to the advantages they offer. Since they do not contain a fluid phase needed for induction into the lungs and nose, they are characterized by an increased chemical and microbiological stability, without the necessity of including preservatives (13,26). Dry powders are developed using spray-drying and freeze-drying techniques (27). In the formulation process, the cohesion between aerosol particles is an essential factor to be considered and prevented, usually by the addition of "carriers" such as lactose or leucine. By

their inclusion to the compositions, the aggregation between small particles is overcome, and the size is kept in an optimal range during the formulation process and during storage, facilitating drug deposition into the lungs (16,28). The in-process control of the dry powder inhalation dosage forms includes particle size analysis, size distribution, powder X-ray diffraction, compatibility studies, and microscopic evaluation of the particle surface (29,30).

Therapeutic Impact of Aerosol Vaccines

Different clinical studies have been conducted to evaluate the effectiveness of aerosol vaccines in case of diseases such as measles, tuberculosis, influenza, anthrax, or Ebola virus disease (31-35).

Measles is an airborne infectious disease caused by a measles virus. The most distinctive symptom of this condition is the flat red rash densely spread over the face and body of the infected (31). Measles is reported to be highly contagious, especially for non-immune people. Although it is an avoidable disease, an object of numerous vaccination programs, it is still a massive cause of mortality, reaching an unreasonable peak in 2015, manifested in 134 200 deaths worldwide (36). In 2002, the World Health Organization (WHO) founded a program named Measles Aerosol Project, which carried out clinical and non-clinical studies to evaluate the potential of an aerosol vaccine against the disease. The outcomes from the conducted studies reported that the aerosol vaccine was safe, well-tolerated, and non-immunogenic (37). Similar results were obtained by various studies performed worldwide (8,25). Amor *et al.* carried out a randomized trial on first-grade children in Mexico and reported that the tested aerosol vaccine was more immunogenic for measles antibodies and equally immunogenic for rubeola antibodies in comparison to a subcutaneous vaccine (8). Another study among schoolchildren in Mexico also confirmed the superior immunogenicity of aerosol measles vaccine compared to a subcutaneous one manifested with antibody level of 52-64% in the aerosol vaccinated group compared to 4-23% in the injection vaccine group (25).

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* mainly affecting the lungs (38). Pulmonary tuberculosis is a cause of millions of deaths yearly, even though a reliable vaccine exists – Bacille Calmette-Guérin (BCG), established in the 1920s (12). When injected after birth, BCG protects against disseminated pediatric tuberculosis; however, protection in adults is reported to be volatile (32). New strategies are elaborated, aiming to improve the BCG effect using vectors as carriers for *M. tuberculosis* antigens (32). According to studies performed on mice, the aerosol and nasal sprays showed better protection against *M. tuberculosis* than parental application (39). Results of a phase I clinical study, conducted in the UK, showed an improved efficacy, expressed in higher antibody levels reached by aerosol vaccination (using mesh nebulizers) compared to an intradermal one (40).

Influenza is a significant cause of mortality for chronically diseased and immunosuppressed patients. Annually new parental vaccines are developed due to the continuously changing antigen variations. Limited induction of local immunity may be noted as their main drawback (2). Thereupon intranasal vaccines are a promising approach since they are reported to increase local drug bioavailability to the mucosa tissue (13). A study performed on rats investigated the efficacy of the aerosol vaccine consisting of spray-dried influenza antigens and bio-lipids, existent in the pulmonary surfactant as excipients, compared to the subcutaneous injections. The obtained results showed that the tested vaccines mainly induce the specific IFN-γ producing T cells and increase the synthesis of IL-2 in the spleen, ensuring increased efficiency due to developed immunity at the port of the entry (13). According to the outcomes from phase III clinical trials, improved effectiveness of intranasally applied influenza vaccine (Flumist) was reported in children compared to a conventional trivalent inactivated vaccine (33).

Besides the diseases mentioned above, the potential use of aerosol vaccines was also investigated regarding the Ebola virus disease and anthrax prevention. In their study Meyer *et al.* tested on primates parainfluenza virus type 3 respiratory vaccine containing glycoprotein of the Ebola virus, which induced improved immunogenicity and specific T-cell response in the lungs compared to systemic replicon vaccine (35). Since the presence of anthrax spores remains in the lungs after exposure, various studies investigate the effect of aerosol vaccines for the prevention of the disease (41). In their research, Gaur *et al.*

reported the improved immunogenicity of intranasally applied recombinant protective antigen-based anthrax vaccine in mice compared to subcutaneous ones (34). Regarding the formulation factors, greater effectiveness of the tested vaccine was determined when the protective antigen was intranasally applied in powder form than as a liquid (42).

Recent Advances in Aerosol Vaccines

Pathogens such as SARS-CoV, MERS-CoV, and SARS-CoV-2 have challenged scientists from all over the world in search of vaccines to treat virus-induced diseases. Although the first cluster of SARS cases occurred in China's Guangdong province in November 2002, there is still no specific treatment and approved vaccine. The earliest case of MERS dates back to April 2012, and to date, there is no approved vaccine or specific treatment, although the case fatality rate is 34.8%. The first cases of COVID-19 were reported in Wuhan, China, in December 2019, and two months later, in February 2020, the World Health Organization (WHO) declared a pandemic (43). Today the confirmed cases of COVID-19 are about 3.5 million in nearly 200 countries globally, and the number of infections and deaths will continue to rise (44). In the face of this gloomy outlook, researchers around the world have joined forces searching and identifying suitable treatments and vaccines to curb the pandemic (45). Several research teams considered the nasal route of administration as appropriate for COVID-19 vaccine candidates. The aim of researchers from the University of Waterloo in Canada is to deliver the DNA-based vaccine to cells in target tissues and trigger the production of virus-like particles (VLP) that will induce an immune response (46). Furthermore, scientists from the University of Eastern Finland are also developing a nasal COVID-19 vaccine based on gene transfer technology (47).

CoroFlu is a unique nasal vaccine against COVID-19 that is built on a flu vaccine "backbone" (48). CoroFlu is under development by a collaboration between the University of Wisconsin-Madison and the US vaccine company FluGen and Hyderabad-based Bharat Biotech (49).

AdCOVIDTM, a single-dose nasal vaccine in clinical trials, has the potential to stimulate multiple arms of the immune system. Altimmune Inc, in collaboration with the University of Birmingham, AL, US, utilizes its proprietary intranasal vaccine technology (NasoVAXTM, an influenza vaccine candidate based on the same platform technology; Phase 2 clinical study) in the new AdCOVIDTM (50).

Recently, APEPTICO Forschung und Entwicklung GmbH, in collaboration with the Medical University Vienna, announced that Solnatide is into phase II placebo-controlled randomized study aiming to explore the efficacy of Solnatide IMP in SARS-CoV-2 positive patients with moderate-to-severe ARDS and pulmonary permeability edema (51).

Moreover, Neurimmune AG, Zurich, Switzerland, and Ethris GmbH, Munich, Germany, have partnered to develop an inhaled mRNA-encoded vaccine that combines Neurimmune's human antibody development based on high-throughput immunoglobulin sequence analyses from Covid-19 patients who have recovered from the disease and on Ethris' pulmonary SNIM®RNA therapeutics platform (49,52).

Aerosol vaccines, utilizing novel technologies in drug delivery, seem to be a promising approach in the prevention and treatment of the SARS-CoV-2 virus-induced diseases.

CONCLUSION

Aerosol vaccines are a feasible alternative to conventional ones, characterized by their ability to provide local immunity, high patient compliance, safety profile, and according to the results from conducted clinical trials - comparable or improved efficiency. Future aspects of aerosol vaccine development include the necessity of further research to evaluate their full potential as well as technological and economic optimization of the formulation process, which will further facilitate their widespread clinical use.

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