Advanced Drug Delivery Reviews 65 (2013) 782–799

Contents lists available at SciVerse ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Oral delivery of human biopharmaceuticals, autoantigens and vaccine antige bioencapsulated in plant cells☆

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ARTICLE INFO

Article history:
Accepted 17 October 2012
Available online 23 October 2012

Keywords:
Autoantigens
Bioencapsulation
Diabetes
Hemophilia
Infectious diseases
Lyophilization
Molecular farming
Oral vaccines

ABSTRACT

Among 12 billion injections administered annually, unsafe delivery leads to >20 million infections and >100 million reactions. In an emerging new concept, freeze-dried plant cells (lettuce) expressing vaccine antigens/biopharmaceuticals are protected in the stomach from acids/enzymes but are released to the immune or blood circulatory system when plant cell walls are digested by microbes that colonize the gut. Vaccine antigens bioencapsulated in plant cells upon oral delivery after priming, conferred both mucosal and systemic immunity and protection against bacterial, viral or protozoan pathogens or toxin challenge. Oral delivery of autoantigens was effective against complications of type 1 diabetes and hemophilia, by developing tolerance. Oral delivery of proinsulin or exendin-4 expressed in plant cells regulated blood glucose levels similar to injections. Therefore, this new platform offers a low cost alternative to deliver different therapeutic proteins to combat infectious or inherited diseases by eliminating inactivated pathogens, expensive purification, cold storage/transportation and sterile injections.

Contents

Abbreviations: APC, antigen presenting cell; AMA, apical membrane antigen-1; BCG, Bacille Calmette Guerin; CDC, Centers for Disease Control and Prevention; CTB, cholera toxin B subunit; DC, dendritic cell; EX4, exendin-4; FIX, blood coagulation factor IX; GAD, glutamate decarboxylase; GIT, gastrointestinal tract; GLP-1, glucagon-like peptide-1; ID, intradermal injection; IPV, inactivated poliovirus vaccine; LT-B, heat labile enterotoxin B subunit; MSP1, merozoite surface protein-1; NOD, non-obese diabetic; ORV, orally immunized mice; PA, protective antigen from *Bacillus anthracis*; SC, subcutaneous injection; SQV, subcutaneously immunized mice; TLP, total leaf protein; TSP, total soluble protein.

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Nanoparticle- and biomaterials-mediated oral delivery for drug, gene, and immunotherapy".

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http://dx.doi.org/10.1016/j.addr.2012.10.005

1. Introduction

Infectious diseases pose the greatest threat for human lives. Production of cost effective, safe and easily deliverable vaccines against current and emerging infectious diseases is needed. The morbidity and mortality rate due to infectious diseases are very high in developing countries and every year > 9.5 million people die [1], despite development of vaccines. This is because of several limitations of current vaccines including their safety, complex production methods that require prohibitively expensive fermentation systems, purification systems, cold storage, cold transportation and sterile delivery [2]. Vaccination is effective to prevent infectious diseases but in developing nations its use is limited by higher cost [2]. In addition, vaccine delivery requires licensed health practitioners and injectable drugs could get contaminated during storage or their dispensation. Physical discomfort of injections also decrease patient compliance.

Similarly, conventional delivery of therapeutic proteins utilizes sterile needle and syringe. Compliance is one of the most important factors, especially, among diabetic patients who may take more than 60,000 injections during their life time [3]. Although new technologies for the parenteral delivery of insulin, such as insulin injection port, pump, jet injector, and implantable pumps provide new alternatives to daily injections, there have been several disadvantages, including skin irritation, erythema, abscess formation, scarring, and site infections [4]. Currently, the commercial production of therapeutic proteins/vaccines is mainly dependent on bacterial, yeast or mammalian cell expression systems. The production also requires expensive purification steps, cold storage and additional delivery cost to patients, contributing to higher cost of drugs.

Parenteral injections via intramuscular, intravenous, intradermal, and other routes are widely used for delivery of therapeutic proteins and vaccine antigens. According to WHO, 12 billion injections are used globally each year including 600 million vaccinations and 11.4 billion for other treatments [5]. Unsafe medical injections have led to 15 million HBV infections, 1 million HCV infections, 340,000 HIV infections, 3 million bacterial infections and 850,000 injection site abscesses [6]. Parenteral vaccination has another immense disadvantage of generating predominantly systemic immune response with little or no mucosal immunity [7]. So the development of a more convenient and cost-effective delivery system has long been desired. There have been a great number of investigations on efficient delivery of therapeutic proteins/vaccines to patients who are dependent on long-term treatments. In this article, we review the literature on various delivery methods currently used or in development for administration of biopharmaceuticals, autoantigens and vaccines or vaccine antigens. The advantages and disadvantages of each method are discussed using specific examples.

Most importantly, we describe a new concept for oral delivery of therapeutic proteins, autoantigens and vaccine antigens expressed and bioencapsulated in plant cells. Freeze-dried plant cells (lettuce) expressing vaccine antigens or biopharmaceuticals are protected in the stomach from acids and enzymes but are released to the immune or blood circulatory system when plant cell walls are digested by microbes that colonize the gut. Oral boosters of vaccine antigens after priming confer both mucosal and systemic immunity and protection against pathogen or toxin challenge. Oral delivery of autoantigens confers tolerance and relief against allergic immune responses. Oral delivery of biopharmaceutical proteins confer desired functions (e.g. regulation of blood glucose levels).

This technology facilitates long-term storage of vaccines at room temperature and eliminates: cold storage, transportation, inactivated or attenuated pathogens and expensive fermentation, purification and sterile injections. Plant based expression system offers several unique advantages including low cost of manufacturing, easy scale up, minimal risk of contamination with human pathogens or toxins that are encountered in bacterial, yeast or mammalian cell culture systems. Three types of plant expression systems are currently used: transient plant viral system and stable expression via the plant nuclear or chloroplast genomes [2]. Among these, the plant chloroplast system is particularly suitable for oral delivery because of high levels of expression (up to 70% of the total leaf protein) of therapeutic proteins [8], thereby facilitating large scale production (up to 300 million doses per acre) or delivery of appropriate doses for the human body [9,10]. In addition, harvest of leaves before emergence of reproductive structures and maternal inheritance of transformed chloroplast genomes containing genes coding for therapeutic proteins offer several layers of biological containment of transgenes [11,12]. Such containment addresses one of the major environmental concerns in using genetically modified plants. In addition, multigene engineering is feasible in a single step [13–15], facilitating parallel expression of several subunits of vaccine antigens [16–18] or biopharmaceuticals [8,19,20]. Therapeutic proteins expressed in chloroplasts form disulfide bonds and are properly folded and fully functional [20–23].

The general process for expression, bioencapsulation, lyophilization, preparation of capsules and evaluation of functionality of biopharmaceutical proteins, vaccine antigens or autoantigens, delivered by injections or oral gavage is described in Fig. 1. Foreign genes are first expressed in lettuce chloroplasts by bombardment of leaves with chloroplast vectors using the gene gun. After confirmation of stable integration of foreign genes into all of the chloroplast genomes in each plant cell — up to 10,000 copies of transformed genomes per cell [24] and absence of any native chloroplast genome and expression of the correct size fully functional protein, genetically modified lines are transferred to the greenhouse for increasing biomass. Harvested leaves are lyophilized, powdered and stored in moisture free environment at room temperature. Machines are commercially available for processing lyophilized leaf materials into desired particle size and packaging into capsules. Lyophilization process also eliminates microbes that are usually present within intercellular spaces in fresh leaves. Therapeutic proteins maintain their integrity, folding (with disulfide bonds, pentameric or multimeric structures) for several months or years when stored at room temperature [25] and functionality (by conferring immunity with vaccine antigens or developing tolerance with autoantigens or regulating blood glucose with insulin, exendin-4, etc.). In this review, we give selected examples of oral delivery of biopharmaceutical proteins (insulin, exendin-4), autoantigens (diabetes, hemophilia) or vaccine antigens (cholera, malaria, plague) bioencapsulated in plant cells and evaluation of their efficacy when compared with the injectable delivery system.

2. Current delivery methods for biopharmaceutical proteins and vaccines

2.1. Parenteral delivery

The commonly used parenteral methods for vaccine delivery are subcutaneous, intramuscular, intradermal, intraperitoneal and intravenous