International Journal of Pharmaceutics 529 (2017) 1–14

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Nasal vaccination with poly(β-amino ester)-poly(D,L-lactide-*co*-glycolide) hybrid nanoparticles

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ARTICLE INFO

Article history:
Received 26 April 2017
Received in revised form 13 June 2017
Accepted 15 June 2017
Available online 16 June 2017

Keywords:
Mucosal vaccination
Nasal delivery
Nanoparticle
Poly(β-amino ester)
Poly(D,L-lactide-*co*-glycolide)
Antibody
Cytokine

ABSTRACT

Mucosal vaccination stimulates both mucosal and systemic immunity. However, mucosal applications of vaccine antigens in their free form generally result in poor systemic immune responses and need adjuvantation. In this study, bovine serum albumin loaded, new hybridised poly(β-amino ester)-poly(D,L-lactide-*co*-glycolide) nanoparticles were prepared by double emulsion-solvent evaporation method, characterised and evaluated *in vivo* as nasal vaccine carriers. Cationic spherical particles with a mean size of 240 nm, good physical stability and high encapsulation efficiency were obtained. Protein structure was not affected throughout preparation and minimal toxicity was shown in Calu-3 and A549 cells. Nasal vaccination with these nanoparticles revealed markedly higher humoral immune responses compared with free antigen following intranasal and subcutaneous immunisation. Mucosal immune response was also stimulated and cytokine titres indicated that Th1 and Th2 pathways were successfully activated. This study shows that the formulated hybrid nanoparticles can be a promising carrier for nasal immunisation of poor antigenic proteins.

1. Introduction

Vaccination is widely considered as one of the greatest breakthroughs in modern medicine. Many lives have been saved and quality of life has been improved through the use of vaccines. The challenge to develop novel, safe and efficient antigen delivery systems remains still an urgent need not only for the ever increasing number of new emerging vaccine antigens that are coming, but also for the improvement of efficacy, safety and ease of administration of already existing vaccines.

Alternative route to parenteral vaccination, mucosal route has great potential since application through mucosa can stimulate both mucosal and systemic immunity. In particular, the nasal cavity with its easy accessibility and its large absorption area represents a potential portal for the delivery of vaccines. Moreover, specialized antigen-sampling microfold cells (M cells) in nasal epithelium layer can mediate strong local and systemic immune responses (Sharma et al., 2009). However, mucosal applications of vaccine antigens in their free form generally result in poor systemic immune responses. To combat this weakness, the use of polymeric particulate delivery systems has been found to improve the immunogenicity of mucosal vaccines and enhance antigen uptake (Cevher et al., 2015a; Thiele et al., 2001). Encapsulation of antigens into particulate carriers not only can protect them from the enzymatic degradation at the mucosal surface and improve their uptake and translocation *via* the epithelial cells and M cells, but also prolong the release of entrapped antigens, thereby leading to stronger and longer lasting immune responses (Corr et al., 2008; Taki and Smooker, 2015).

Food and Drug Administration (FDA) approved poly(D,L-lactide-*co*-glycolide) (PLGA) has been commonly used in the preparation of micro-/nanoparticles. The potential of biodegradable and biocompatible nanoparticles prepared from the PLGA polymer as a safe

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http://dx.doi.org/10.1016/j.ijpharm.2017.06.053
0378-5173/

and effective vaccine delivery system for a variety of antigens has been shown in numerous studies (Li and McCarthy, 1999; Tamber et al., 2005; Zhao et al., 2014b). Many investigations that have been conducted in this area, including our studies, have demonstrated that particles both in micron and nano dimensions can cross epithelial membrane barrier following intranasal (i.n.) administration (Alpar et al., 1994; Csaba et al., 2006). For example, encapsulation of Hepatitis B surface antigen (HbSAg) into PLGA/poly(L-lactic acid) (PLA) particles enhanced both systemic and mucosal immune responses following nasal delivery (Alpar et al., 2001; Jaganathan and Vyas, 2006). Despite the promising results coming from these studies, PLGA particles formulated so far do not encompass all the necessary properties for achieving the desired clinical efficacy, although many exhaustive work has been done to optimise their physicochemical characteristics to function as effective vaccine carrier. The inherent weak mucoadhesiveness of PLGA per se leading to relatively rapid nasal clearance has directed the attention towards surface modification with cationic polymers to improve residence time of the carrier and antigen in the nasal cavity. Numbers of investigations have reported that coating of the surface of PLGA particles with positively charged polymers enhanced immune response *via* nasal route (Pawar et al., 2010, 2013; Zhao et al., 2014a).

Poly(β-amino esters) (PbAEs) are easy to synthesize, pH sensitive, hydrolytically degradable cationic polymers which can be good candidates for improving adjuvanticity of native PLGA particles (Fields et al., 2012). These polymers are synthesised via Michael addition reaction of amine monomers and diacrylates (Chen et al., 2007). PbAE polymers of different kinetic degradation profile and positive charge density have been synthesised using various amine and acrylate derivatives and gained huge interest as new drug delivery systems (Lynn et al., 2001). Up to date, successful results have been obtained with these polymers on drug delivery and genetic vaccination studies (Little et al., 2004; Shenoy et al., 2005). Andorko et al. (2016) confirmed the intrinsic immunogenicity of PbAE particles and ability to activate dendritic cells (DCs). Although the potency of PbAE-PLGA nanoparticles as delivery system for DNA vaccination has been investigated, there are no prior reports for the use of these particles as vaccine carrier systems for protein/peptide antigens that are delivered via the nasal route.

In this work, we describe the formulation and optimisation of a hybrid PbAE-PLGA nanoparticulate system and evaluate its potential as nasal antigen carrier. The hybrid carrier is intended to combine the benefits of cationic PbAE polymer with regard to mucoadhesiveness, high drug loading capacity and efficient cellular uptake with the physicochemical characteristics of PLGA nanoparticles. Bovine serum albumin (BSA) was chosen as the model antigen for this study and adjuvanticity of formulated PbAE-PLGA hybrid nanoparticles was evaluated both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-*co*-glycolide) (PLGA 50:50) Resomer® RG502 (7000–17000 Da) and Resomer® RG503 (24000–38000 Da) were purchased from Evonik, Germany. Bisphenol-A-ethoxylate diacrylate, ethylenediamine, tetrahydrofuran, bovine serum albumin (BSA) (~66 kDa), poly(vinyl alcohol) (30000–70000 Da, %89–97 hydrolysed), QuantiPro™ Bicinchoninic Assay Kit, sodium azide, Dulbecco's modified Eagle's medium (DMEM), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), fetal bovine serum (FBS), cell culture freezing medium, trypsin-EDTA, were purchased from Sigma-Aldrich, Germany. Cell strainer (70 μm) and cell culture flasks were purchased from SPL, South Korea. Alum and CpG ODN were purchased from Invivogen, France. Eagle's minimum essential medium (EMEM) and RPMI 1640 were purchased from Multicell, USA and Lonza, Belgium, respectively. Penicillin/streptomycin was purchased from Gibco, USA. Phosphate buffered saline (PBS) tablet (pH 7.4) was purchased from Genaxxon Bioscience GmbH, Germany. 96-well cell culture plate was purchased from Costar, USA. ELISA General Assay Diluent, ELISA wash buffer and stop solution were purchased from Biolegend®, USA. Laemmli sample buffer, β-mercaptoethanol, 10X Tris/Glycine/SDS buffer, Goat Anti Mouse IgG:HRP, Goat Anti Mouse IgG_1:HRP, Goat Anti Mouse IgG_{2a}:HRP, Goat Anti Mouse IgA:HRP were purchased from Bio-Rad, USA. Flat bottom 96-well ELISA plates were obtained from Thermo Scientific, USA. Coomassie Brillant Blue R250 was purchased from Glemtham Life Sciences, UK. 2,2′-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS) solution, dimethyl sulfoxide (DMSO), diethyl ether, acetic acid, dichloromethane and all other chemicals were of analytical grade and were purchased from Merck, Germany. Deionised water was used to prepare all solutions and buffers.

2.2. Synthesis of poly(β-amino ester) polymer

PbAE polymer was synthesised in house by Michael addition reaction between bisphenol-A-ethoxylate diacrylate and ethylenediamine (Fig. 1) and characterised as described in patent application of our group (data not given) (Gök et al., 2016).

2.3. Preparation of BSA loaded poly(β-amino ester)-poly(D,L-lactide-co-glycolide) hybrid nanoparticles

BSA loaded PbAE–PLGA hybrid nanoparticles (PbAE-PLGA-BSA) were prepared by water-in-oil-in-water (W/O/W) double emulsion solvent evaporation method (Fig. 2). Briefly, a primary emulsion (W/O) was formulated by adding dropwise 500 μL of

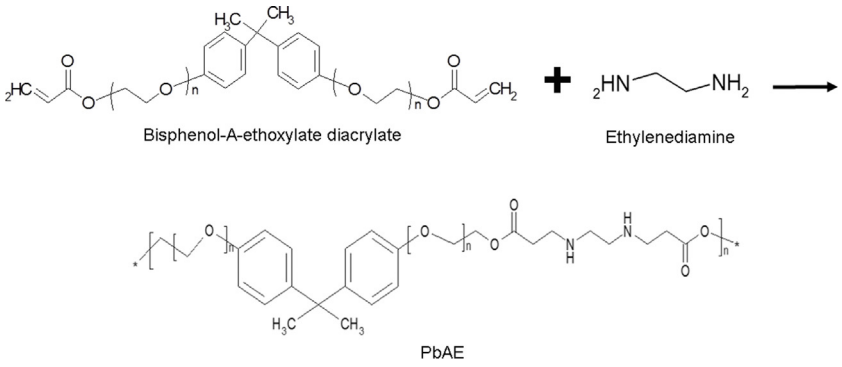

Fig. 1. Synthesis of poly(β-amino ester) polymer.