Chemical Engineering Science 125 (2015) 98–107

Contents lists available at ScienceDirect

Chemical Engineering Science

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

Sustained release and stabilization of therapeutic antibodies using amphiphilic polyanhydride nanoparticles

Brenda R. Carrillo-Conde [a], Ross J. Darling [b], Steven J. Seiler [a], Amanda E. Ramer-Tait [b,c], Michael J. Wannemuehler [b], Balaji Narasimhan [a,*]

[a] Department of Chemical and Biological Engineering, Iowa State University, Ames, IA 50011, United States
[b] Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, United States
[c] Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE 68583, United States

HIGHLIGHTS

- Antibody therapy is limited by antibody instability and short in vivo half-life.
- Amphiphilic polyanhydride nano-carriers released biologically active antibodies.
- Nano-carriers enabled sustained antibody release, thus prolonging in vivo half-life.
- This approach will lead to enhanced patient compliance and cost effective therapies.

ARTICLE INFO

Article history:
Received 6 May 2014
Received in revised form
5 August 2014
Accepted 8 August 2014
Available online 14 August 2014

Keywords:
Polyanhydrides
Nanoparticles
Therapeutic antibodies
Delivery
Stability

ABSTRACT

Passive administration of antibodies (e.g., anti-serum or monoclonal antibodies) can be successfully used as treatments for infectious agents (e.g., human cytomegalovirus, HIV), chronic inflammation (e.g., anti-TNF), cancer (e.g., anti-HER2, anti-VEGF, anti-CD20), toxins (e.g., anti-ricin), and age-related diseases such as macular degeneration (e.g., anti-VEGF). As with the development of proteinaceous pharmaceuticals, one of the most challenging obstacles facing passive immunotherapies is the physical and the chemical instabilities of the antibodies, which invariably leads to loss of biological activity. In order to avoid these problems, appropriate delivery vehicles need to be designed that minimize the degradation, maximize the in vivo activity, and provide controlled release of the encapsulated biologically active protein. In this study, polyanhydride nanoparticles were used for the delivery of stable and biologically active therapeutic antibodies. Tetanus antitoxin and anti-TNF-α monoclonal antibodies were encapsulated and released from polyanhydride nanoparticles. The nanoparticles provided the ability to control antibody release kinetics and, additionally, preserved antibody functionality and bioactivity upon synthesis and release. In particular, amphiphilic polyanhydride nanoparticles demonstrated the best combination of characteristics compatible for anti-serum or monoclonal antibody preservation and release, making them ideal candidates for use as a delivery system for therapeutic antibodies.

1. Introduction

The sale of biopharmaceutical therapeutics is estimated to account for about 15% of the entire pharmaceutical market (Hahn, 2009). With over 300 monoclonal antibody (mAb) therapeutics under development and more than 25 already approved for human use, antibody therapeutics represent, based on worldwide sales, the largest segment of the biotherapeutics market (Hahn, 2009; Piggee, 2008; Presta, 2002).

Antibodies against microbial, cellular and soluble targets exploit various effector functions such as opsonization, activation of complement, antibody-dependent cell cytotoxicity, and virus and toxin neutralization for therapeutic purposes (Casadevall et al., 2004; Grainger, 2004). Antibody therapies have been developed for a wide range of disease conditions in oncology, inflammation, immune-mediated disorders, and wound healing (Casadevall et al., 2004; Grainger, 2004; Nelson et al., 2010). However, serum half-life of a passively transferred antibody can be between 2 and 15 days, requiring long-term, repetitive high dosing, which is the bane of antibody therapy (Mould and Sweeney, 2007; Wang et al., 2007). Therefore, the development of sustained delivery vehicles would significantly benefit drug bioavailability and patient compliance

* Corresponding author. Tel.: +1 515 294 8019; fax: +1 515 294 2689.
E-mail address: nbalaji@iastate.edu (B. Narasimhan).

http://dx.doi.org/10.1016/j.ces.2014.08.015
0009-2509/

(Nelson et al., 2010; Scweizer et al., 2013). The development of formulations for parenteral administration and controlled delivery of protein drugs is particularly challenging, resulting in a lack of commercially available sustained delivery systems for antibodies and a limited number of studies on this topic (Grainger, 2004; Guziewicz et al., 2011; Saltzman, 1993; Salzman et al., 1993; Scweizer et al., 2013).

In this regard, it is useful to consider biodegradable polymer micro- and nanoparticles that have been designed as sustained delivery vehicles for biologically active proteins such as antibodies. The advantage of using polymeric particles lies in their customized design to direct local delivery at diseased sites and control the protein release kinetics profile. However, there are still several drawbacks in the design of these polymeric devices for protein delivery, including protein–material incompatibilities such as hydrophobicity differences that commonly result in poor loading efficiency and unfavorable microenvironments caused by (acidic) polymer degradation products, which occurs during the degradation of poly(lactic-co-glycolic acid) (PLGA) (Grainger, 2004; Zhu et al., 2000). Additional challenges include the processes typically used (i.e., organic solvents, extreme pH, and mechanical stress) for the synthesis of these particles and the poor encapsulation of protein therapeutics within. Exposing antibodies to these conditions often results in irreversible chemical and/or physical instabilities (Wang, 1999). In order to achieve good therapeutic efficacy, it is necessary to ensure that the polymer chemistry and the particle synthesis methods do not compromise antibody function.

While several biomaterials have been used for antibody delivery applications, polyanhydride particles have the potential to address the challenges described above. Biodegradable polyanhydride particles have high biocompatibility, controllable degradation rates due to their surface erosion degradation mechanism, and a favorable microenvironment resulting from their less acidic degradation products (Determan et al., 2004; Kipper et al., 2002, 2006; Lopac et al., 2009; Torres et al., 2007; Torres et al., 2011; Ulery et al., 2011a). Specifically, polyanhydride micro- and nanoparticles have been successfully used for sustained delivery and stabilization of several protein-based vaccines (Carrillo-Conde et al., 2010; Determan et al., 2006a; Haughney et al., 2013; Petersen et al., 2012; Ross et al., 2014; Torres et al., 2007; Ulery et al., 2011a, 2011b).

The current work investigated the use of polyanhydride nanoparticles for the delivery of two therapeutic antibodies: tetanus antitoxin, an antiserum used for the treatment of equine tetanus (Blake et al., 1976; Lang et al., 1993), and anti-TNF-α, a mAb used to treat Crohn's disease and rheumatoid arthritis (Casadevall et al., 2004; Havell and Rogerson, 1993; Presta, 2002). Polyanhydride particle formulations were designed to optimize antibody loading, release kinetics, and stability. A systematic structural analysis of released antibody from polyanhydride nanoparticles was performed, which provided important insights into the mechanisms governing polyanhydride–antibody interactions. Finally, the ability of polyanhydride nanoparticles to deliver anti-TNF-α mAb in vivo that maintained the neutralizing capacity in serum was demonstrated. These studies demonstrate that amphiphilic polyanhydride nanoparticles preserved antibody functionality and suggest that this platform can be effectively used as an in vivo delivery system for therapeutic antibodies.

2. Materials and methods

2.1. Materials

Materials needed for monomer synthesis and polymerization, as well as for nanoparticle synthesis, including sebacic acid (99%), *p*-carboxy benzoic acid (99+%), and 1-methyl-2-pyrrolidinone, anhydrous (99+%), were purchased from Aldrich (Milwaukee, WI); 4-*p*-hydroxybenzoic acid, 1,6-dibromohexane, 1-methyl-2 pyrrolidinone, and tri-ethylene glycol were purchased from Sigma Aldrich (St. Louis, MO); 4-*p*-fluorobenzonitrile was obtained from Apollo Scientific (Cheshire, UK); potassium carbonate, dimethyl formamide, toluene, sulfuric acid, acetic acid, acetonitrile, acetic anhydride, methylene chloride, pentane, and petroleum ether were purchased from Fisher Scientific (Fairlawn, NJ). Phosphatase substrate was purchased from Aldrich (St. Louis, MO). Polyacrylamide 4–20% Tris–HCl pre-cast gradient gels, unstained protein standards, pre-stained broad range molecular weight standards, and Flamingo Gel Stain were purchased from BioRad Laboratories (Richmond, CA). Bicinchoninic acid (BCA) and microBCA protein assay kits and Slide-A-Lyzer dialysis cassettes (10,000 MW cut-off membranes) were obtained from Pierce Biotechnology Inc. (Rockford, IL).

2.2. Monomer and polymer synthesis

Diacids of 1,6-bis-(*p*-carboxyphenoxy)hexane (CPH) and 1,8-bis-(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) were synthesized as described previously (Conix, 1966; Torres et al., 2006). Sebacic acid (SA) and CPH prepolymers were synthesized by the methods described by Shen et al. (2001) and Conix (1966), respectively. Subsequently, 20:80 CPH:SA and 50:50 CPTEG:CPH copolymers were synthesized by melt polycondensation as described by Kipper et al. (2002) and Torres et al. (2006), respectively. The chemical structure of the polymer was characterized with ^{1}H NMR and the molecular mass was determined using gel permeation chromatography (GPC) (Kipper et al., 2002; Torres et al., 2006).

2.3. Nanoparticle synthesis

Nanoparticles encapsulating 2% (w/w) of lyophilized antibodies were synthesized by anti-solvent nanoencapsulation (Ulery et al., 2009). The antibodies utilized for the present studies were tetanus antitoxin, a polyclonal equine serum (Zoetis, Fort Dodge, IA) with molecular weight between 150 and 170 kDa depending on the IgG subclass and anti-TNF-α, a rat monoclonal antibody (clone XT3.11, BioXCell Fermentation/Purification Services, West Lebanon, NH) with molecular weight of ~180 kDa (Sheoran et al., 1998). Briefly, polymer was dissolved in methylene chloride (15 mg/mL) held at room temperature for 20:80 CPH:SA and at 4 °C for 50:50 CPTEG:CPH. Tetanus antitoxin or anti-TNF-α mAbs (2% (w/w)) were suspended and homogenized by sonication in the polymer solution prior precipitation. The polymer solution containing the protein was rapidly poured into a bath of pentane held at room temperature for 20:80 CPH:SA and −40 °C for 50:50 CPTEG:CPH at an antisolvent to solvent ratio of 1:150. Particles were collected by filtration and dried under vacuum for 2 h. Nanoparticle morphology and size distribution were characterized by scanning electron microscopy (SEM, JEOL 840A, JEOL Ltd., Tokyo, Japan) and quasi-elastic light scattering (QELS, Zetasizer Nano, Malvern Instruments Ltd., Worchester, UK), respectively.

2.4. In vitro antibody release

Tetanus antitoxin and anti-TNF-α loaded nanoparticles were placed in low-protein binding microcentrifuge tubes and suspended in one milliliter of 0.1 mM phosphate buffer saline (PBS, pH 7.6). Samples were sonicated briefly to uniformly distribute the nanoparticles and the microcentrifuge tubes were placed in an incubator at 37 °C with constant agitation. Supernatants were sampled over time to determine the amount of released antibody using the microBCA protein quantification assay. Removed