International Journal of Pharmaceutics 487 (2015) 234–241

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Investigation of 3D ordered macroporous carbon with different polymer coatings and their application as an oral vaccine carrier

Qinfu Zhao [a], Qiang Zhang [a], Yang Yue [a], Jiahao Huang [a], Donghua Di [a], Yikun Gao [b], Xinyi Shao [c], Siling Wang [a,*]

[a] Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang, Liaoning Province 110016, PR China
[b] School of Medical Devices, Shenyang Pharmaceutical University, PR China
[c] Department of Traditional Chinese Medicine, College of Traditional Chinese Medicine, Shenyang Pharmaceutical University, PR China

ARTICLE INFO

Article history:
Received 16 February 2015
Received in revised form 2 April 2015
Accepted 16 April 2015
Available online 17 April 2015

Keyword:
3-D ordered macroporous carbon
Chitosan
Polyethyleneimine
Oral immunity
Release rate
Electrostatic attraction

ABSTRACT

3-D ordered macroporous carbon with different polymer coatings were developed as new oral vaccine immunological systems. Poly dimethyl diallyl ammonium (PDDA), polyethyleneimine (PEI) and chitosan (CTS), three different polymers with electropositive or adsorption-promoting properties, were chosen as the coating materials to endow the vaccine delivery systems with different surface properties. The bovine serum albumin (BSA) was used as a model vaccine. The three different polymer coated systems exhibited similar release rate which minimized the influence of release rate. The measured value of immunoglobulin G (IgG) titers suggested that the sustained release rate of BSA from polymer coated systems exhibited no strengthened effect on the immune response but could delay the appearance of the peak of the IgG titers compared with uncoated system. The electrostatic attraction between the mucosal and positively charged carrier would be useful during the whole immune experiment. In addition, using the coating material with the ability of enhancing mucosal adsorption was important in the mid-late period of immune. The immunoglobulin A (IgA) titers induced by the polymer coated systems were significantly higher than that induced by the oral BSA solution or i.m. BSA with Freund's complete adjuvant (FCA) which suggested the successful mucosal immune response of the three different coated systems. Overall, this work provides valuable information for the development of oral vaccine delivery system.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Since the application of the vaccines during the past centuries, many incurable diseases have been conquered (Barquet and Domingo, 1997; Stewart and Devlin, 2006; Strassburg, 1982). Due to the significant effect in the anti-infective field, vaccine was considered to be an important breakthrough in medicine. To date, most vaccines are delivered by subcutaneous or intramuscular route. These vaccines can induce systemic immune responses but they are almost useless toward the mucosal immunity.

Among the portals of our body, the mucosa lumen is the main approach for bacterial infections which can be effectively prevented by the body's mucosal immune response (Neutra and Kozlowski, 2006). So inducing the protective immune response just at the site of pathogen entry was an effective method to avoid the mucosa invasion. As a mucosal administration, the gastrointestinal mucosa is the desirable route for protein delivery. Compared to other mucosa lumen, the oral route has the advantages of avoiding pain and reducing the risk of infection. Furthermore, the oral route can induce not only mucosal immunity in the gastrointestinal, but also a robust systemic immune response (Bowersock et al., 1998; Muir et al., 1994). The immunoglobulin A (IgA), which is secreted by the gut-associated lymphoid tissue (GALT) when the vaccine is given orally, can provide early protection for the body. Until now, it is still a very challenging task to design a delivery system which could provide a perfect protection for oral vaccines.

Many nano-vectors have been designed for the oral delivery of proteins (des Rieux et al., 2006; Ma et al., 2014; Thomas et al., 2011; Zhang et al., 2014c; Zolnik et al., 2010). In recent years, the inorganic materials with different pore sizes have been successfully used as an antigen carrier. Compared with some organic materials, the inorganic materials have a stable structure to

* Corresponding author. Tel.: +86 24 23986348; fax: +86 24 23986348.
E-mail addresses: silingwang@syphu.edu.cn, silingwang@hotmail.com (S. Wang).

http://dx.doi.org/10.1016/j.ijpharm.2015.04.045
0378-5173/© 2015 Elsevier B.V. All rights reserved.

overcome the structural instability and the leakage of loaded proteins. In addition, the inorganic materials display many attractive features, such as large surface area, low toxicity, size-adjustable pore size and easily functionalized surface (Li et al., 2004; Zhang et al., 2010).

As an inorganic material, porous carbon has been widely used in biotechnological fields (Wang et al., 2011; Yang et al., 2008). In our previous research (Zhang et al., 2014b), we had succeeded in inducing both the systemic and mucosal immune responses by using 3-D ordered macroporous (pore diameter >50 nm) carbon material (3DC) as a carrier. The advantage of 3DC as our vaccine carrier compared with mesoporous materials was as follows: (1) the inner pore of 3DC was an interconnected space and it was much larger than the loaded protein, so it had huge inner space for protein adsorption, which meant the repository-type carrier had a higher protein adsorption capacity. (2) The release rate of BSA could be regulated by adjusting the amount of protein that adsorbed on the internal/external surface of 3DC. In addition, the rigid framework of 3-D ordered material (3DOM) nanoparticles could have a protecting effect and prevent the protein adsorbed into the carrier from degradation (O'Hagan and De Gregorio, 2009; Zolnik et al., 2010). And the immune results showed that the carrier with a higher adsorption capacity and slower release rate exhibited a higher immune response (Zhang et al., 2014b). Some researchers have reported the combination of organic polymer coating and inorganic materials to adjust the release rate of drugs or proteins (Huang et al., 2011; Sun et al., 2012; Zhang et al., 2014a). In addition, the polymer coating layer could protect the loaded protein form denaturation in the harsh environment of the gastrointestinal tract.

On the basis of previous research, we adopted a strategy of polymer coating for the 3DC carrier and investigated the efficiency in inducing the mucosal and humoral immune response. Three kinds of polymers, Poly dimethyl diallyl ammonium (PDDA), polyethyleneimine (PEI) and chitosan (CTS) were chosen as the coating materials. PEI and PDDA are positively charged polymers and CTS has different charge characteristics in gastrointestinal tract (positively charged in gastric juice and negatively charged in intestinal fluids). In addition, CTS is an effective adsorption enhancer (van der Lubben et al., 2001). The purpose of this paper was to investigate the impact of different coated polymers on the efficiency of inducing mucosal and humoral immunity for the repository-type 3-DC carrier.

2. Materials and experimental conditions

2.1. Materials and animals

Chitosan (CTS, Mw = 100 kDa, degree of deacetylation = 95%) was purchased from HaiDeBei Marine Bioengineering Co. Ltd. (Jinan, China). Poly dimethyl diallyl ammonium chloride (PDDA, Mw = 400–500 kDa), Freund's complete adjuvant (FCA), bovine serum albumin (BSA) and polyethyleneimine (PEI, Mw = 25 kDa) were purchased from Sigma–Aldrich (USA). BCA protein assay kit was obtained from Kangwei Company (Shanghai, China). Goat anti-mouse IgG-HRP was purchased from Zhongshanjinqiao Company (Beijing, China). Goat anti-mouse IgG1-HRP and IgG 2a-HRP were purchased from Proteintech (USA). Goat anti-mouse IgA-HRP was purchased from Santa Cruz (USA). All other chemicals were of analytical grade. All animal studies were approved by the committee of Ethics of Animal Experimentation of Shenyang Pharmaceutical University. All animals were handled and housed under ethical conditions. The female Kunming species BALB/c mice (18–22 g) were provided by the Lab Animal Center of Academy of Military Medical Sciences of the PLA (Beijing, China).

2.2. Synthesis of 3DC materials.

The 3DC material was synthesis using polystyrene (PS) spheres as templates and the synthetic steps were reported in the literature (Holland et al., 1998; Zhang et al., 2009) with some modifications. Briefly, 40 mL styrene (St) was added to an Erlenmeyer flask after washing with NaOH and double distilled water. Then 0.6 g polyvinylpyrrolidone (PVP) K30 was added and the mixture was stirred for 30 min with the temperature raised to 60 °C. Subsequently, 50 mL 0.16 M potassium persulfate (KPS) was added and nitrogen was introduced to exclude oxygen in the system. Then the temperature was adjusted to 70 °C and the system was maintained for 24 h under stirring. The PS spheres were obtained after the reaction solution was centrifuged at 6000 rpm for 40 min. Then the PS spheres templates were dried at 40 °C for 24 h.

13 g sucrose was added to a solution which contained 10 mL H_2SO_4 and 100 mL double distilled water as the carbon precursor-solution. After PS sphere was transferred to a Buchner funnel under vacuum environment, the precursor-solution was added slowly and make sure that it could infiltrate the PS spheres completely. Then the PS sphere was heated and carbonized at 60 °C and 120 °C, respectively. After the carbonization process, the product was calcined at 700 °C under nitrogen atmosphere in order to remove the PS sphere, and the product was named as 3DC. In order to obtain a hydrophilic surface, the 3DC product would undergo a surface modification. In brief, 0.5 g 3DC material was added to a flask and 30 mL 1 M ammonium persulfate solution (dissolved in 2 M H_2SO_4) was added. Then this system was heated to 60 °C with a reflux device and the temperature was maintained 60 °C for 6 h. After the material was filtered and dried, a final product would be obtained and named S-3DC.

2.3. Encapsulation of BSA within S-3DC material.

In this paper, we use the adsorption equilibrium method as our protein adsorption method. Typically, the protein solution was obtained by mixing 14 mg BSA with 7 mL of 20 mM phosphate buffer solution (PBS, pH 7.4) and this solution system was transferred to a refrigerator thermostat with mild stirring for 1 h at 4 °C. Then 10 mg S-3DC material was added and this suspension was stirred at 4 °C. After the adsorption equilibrium for 24 h, the suspension system was centrifuged (8000 rpm for 5 min) and the supernatant was collected and measured by a BCA assay. The amount of BSA adsorbed on the carrier was calculated by subtracting the amount of BSA in supernatant from the total amount of BSA (14 mg).

2.4. Polymer coating on S-3DC/BSA

The preparation process of CTS-coated S-3DC system was as followed: the precursor solution was compounded as followed: 750 μL of 1.5% (v/v%) of acetic acid was added to 50 mL of 0.5 M NaCl solution. Then CTS solution was obtained by dissolving 0.1 g of CTS in the precursor solution with mild stirred for 3 h at 60 °C. Then the CTS precursor solution was cooled down to room temperature for subsequent use. For the encapsulation procedure, 10 mg of BSA-adsorbed S-3DC material was dispersed in 500 μL of 20 mM PBS (pH 7.4) with mild stirring for 1 h at 4 °C. Then 500 μL of the CTS solution was added slowly (dropwise) and stirred for 2 h at 4 °C. Afterward, the suspension was centrifuged at 5000 rpm and washed with double distilled water. This washing step was repeated for three times. After the washing step, the residue was freeze-dried, crushed and passed through a 60 mesh screen. The CTS coated S-3DC was denoted as S-3DC/CTS.

For PEI and PDDA coating, the specific process was same with the CTS coating process. In detail, the precursor solution of PDDA

Download English Version:

https://daneshyari.com/en/article/5818830

Download Persian Version:

https://daneshyari.com/article/5818830

Daneshyari.com