Journal of Controlled Release xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Cationic micelle delivery of Trp2 peptide for efficient lymphatic draining and enhanced cytotoxic T-lymphocyte responses

Qin Zeng [1], Hao Jiang [1], Ting Wang, Zhirong Zhang, Tao Gong, Xun Sun *

Key Laboratory of Drug Targeting and Drug Delivery Systems, Ministry of Education, West China School of Pharmacy, Sichuan University, Chengdu, Sichuan, People's Republic of China

ARTICLE INFO

Article history:
Received 17 June 2014
Received in revised form 3 December 2014
Accepted 17 December 2014
Available online xxxx

Keywords:
Polyethylenimine
Cationic micelle
Trp2 antigen peptide
Draining lymph node
Cytotoxic T lymphocytes

ABSTRACT

Neutral particles 20–45 nm in diameter showed potential as tumor antigen vectors because they targeted the draining lymph nodes after subcutaneous injection. However, they were weakly immune-stimulatory and could also spread throughout the body, raising the risk of systemic toxicity. Here we explored whether incorporating positively charged amphiphilic polymers into micelles improves their site specificity and immunogenicity. Cationic polyethylenimine (2k)-stearic acid (PSA) micelles were loaded with the melanoma antigen peptide Trp2; they showed an average size of 28.7 ± 8.2 nm and an encapsulation efficiency of 99.21 ± 5.38%. Empty PSA micelles acted as a robust adjuvant in vitro, promoting maturation, proliferation and migration of bone marrow-derived dendritic cells in a dose-dependent manner. After subcutaneous injection into mice, Trp2-loaded PSA micelles accumulated preferentially in the medulla and paracortex of the draining lymph nodes and were present at negligible levels in the systemic circulation. Mice immunized with Trp2-loaded PSA micelles showed significantly higher Trp2-specific cytotoxic T lymphocyte activity than mice immunized with free Trp2 or a mixture of Trp2 and empty PSA micelles. In a B16-F10 murine melanoma model, Trp2-loaded PSA micelles inhibited tumor growth significantly more than did free Trp2 and PSA micelles caused less systemic toxicity. These findings suggest that cationic PSA micelles loaded with Trp2 may be a potential approach for melanoma immunotherapy.

1. Introduction

The aim of anticancer immunotherapy is to generate a robust immune response that confers long-lasting protection against tumor invasion, metastasis or recurrence. The success of this approach is reflected in the approval, by the US Food and Drug Administration, of vaccines based on virus-like particles (VLPs) and dendritic cells (DCs) such as Gardasil® against cervical cancer [1] and Sipuleucel-T (Provenge®) against prostate cancer [2]. Despite their promise, these VLPs and other cell-based vaccines, as well as vaccines based on live or attenuated virus, present important disadvantages: they may be associated with undesired side effects and their large-scale production may be difficult. In contrast, vaccines based on short tumor-derived peptides may present several advantages over these viruses, VLPs or cell-mediated vaccines, including greater safety, ease of use and less expensive production on an industrial scale [3]. However, tumor peptide antigens on their own usually fail to induce a protective immune response [3].

Co-administering adjuvants with the peptide antigen could increase the likelihood of a protective and long-lasting immune response. Though the details are poorly understood, adjuvants appear to work through one or multiple several mechanisms, which include prolonging the biological half-life of the antigen as well as enhancing the inflammatory response, recruitment of antigen-presenting cells (APCs) to the site of antigen administration, delivery of antigens to APCs, secretion of immunomodulatory cytokines and chemokines by APCs, and antigen processing and presentation by APCs to other immune cells [4]. Several adjuvants have been approved for use with human vaccines, including Alum, MF59™, MPL, VLPs, immunopotentiating reconstituted influenza virosomes (IRIV), and cholera toxin [5]. Unfortunately most of these adjuvants fail to induce a cell-mediated immune response, and others in development such as Montanides (ISA51 and ISA720) are difficult to formulate or cause unacceptable side effects [5]. Moreover, the wide range of human leukocyte antigen (HLA) epitopes in the population necessitates the parallel development of various vaccines based on the same antigen, exacerbating the need for new, efficient adjuvants [3,4,6].

Persistent T cell immunity using antigen–adjuvant combinations has been achieved by encapsulating them in particles [7]. These particles have been formulated in diverse ways, such as liposomes [8], liposome–polycation–DNA (LPD) [9], emulsions [10], polymers [11] and dendrimers [12]. Antigen–adjuvant particles often have greater effect and induce fewer side effects than the soluble antigen and adjuvant on their own [4,7,13]. This may be because encapsulation protects antigen and adjuvants from degradation, enhances antigen uptake by DCs, improves antigen accumulation in the draining lymph nodes and facilitates its cross-presentation. Some particles have even been shown to act

* Corresponding author at: West China School of Pharmacy, Sichuan University, No. 17, Section 3, Southern Renmin Road, Chengdu 610041, People's Republic of China.
E-mail address: xunsun22@gmail.com (X. Sun).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jconrel.2014.12.024
0168-3659/

as potent adjuvants by themselves [14]. Immunizing mice with particles of (R)-DOTAP carrying Trp2 peptide significantly enhanced cytotoxic T lymphocyte (CTL) activity in vivo compared to those mice given Trp2 alone [15]. While immunogenic, these particles are difficult to modify in order to target them to lymph nodes.

The size and charge of particles strongly influenced their ability to accumulate in the draining lymph nodes and avoid the systemic circulation [16]. For example, the nanoparticles with a diameter of 20–45 nm targeted DCs in draining lymph nodes [17,18]. And PEGylation significantly enhanced 200 nm particles homing to lymph node and uptake by DCs [19]. Both of the small particle size and neutral charge might allow them to travel through interstitial spaces and arrive more easily at the draining lymph nodes [20]. These neutral particles may enhance their systemic dissemination: for example, the liver tropism of PEGylated DOTAP increased with increasing amounts of PEG [21].

As an alternative strategy to reduce systemic dispersion and promote DC maturation and intracellular antigen trafficking within DCs, we wanted to focus on using positively charged particles for delivering tumor antigen peptides. Cationic polyethylenimine (PEI) is widely used as a gene delivery carrier, and it has proven effective as an antigen vector and adjuvant for eliciting protective antibody-mediated and humoral responses to challenge infections with influenza, herpes simplex virus type-2, or HIV-1 [22,23]. Branched 2-kDa PEI (PEI-2k) has been mixed with anionic alginate to form AP-SS nanogels that, after disulfide cross-linking, potently enhanced antibody production and CD8 + T cell-mediated lysis against the model antigen ovalbumin (OVA) [24]. One drawback is that, although widespread as a model antigen, OVA is orders of magnitude more sensitive than the antigens that required to be cross-presented for creating effective vaccines [25].

In this study, the peptide we choose was tyrosine's-related protein 2 (Trp2), the epitope of which is restricted by both of human HLA-A2 and murine major histocompatibility complex (MHC) class I molecule H-2K^b on B16 tumor cells. We further modified branched PEI-2k with stearic acid to produce an amphiphilic molecule (PSA, Fig. 1a). We hypothesized that PSA micelles with a core–shell structure might act a robust adjuvant as well as a preferential antigen vector. We tested the ability of the positively charged PSA micelles loaded with Trp2 to accumulate in the draining lymph nodes and to stimulate antigen-specific CTL activity (Fig. 1b).

2. Materials and methods

2.1. Materials

Branched PEI-2k, 5(6)-carboxyfluorescein diacetate N-succinimidyl ester (CFSE) and lipopolysaccharide (LPS) were obtained from Sigma-Aldrich. Stearic acid, N-hydroxy succinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) were purchased from Aladdin (Shanghai, China), Recombinant mouse granulocyte–macrophage colony-stimulating factor (GM-CSF) was purchased from R&D Systems (Minneapolis, MN, USA). β-Mercaptoethanol and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine (DID) perchlorate were obtained from Life Technologies (Carlsbad, CA, USA).

2.2. Peptide design and synthesis

The peptide H-SVYDFFVWL-OH, corresponding to residues 180–188 of Trp2, was purchased from GL Biochem as an acetate powder (Shanghai, China). The peptide encodes a Trp2 epitope restricted by both human HLA-A2 and the murine major histocompatibility complex (MHC) class I molecule H-2K^b on B16 tumor cells. The disodium salt of Trp2 peptide was formulated as supplementary and their aliquots in 10 mM HEPES (pH 7.2) were stored at −80 °C before use.

2.3. Cell culture and animals

Mouse skin melanoma B16-F10 cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were maintained in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen), 100 U/mL penicillin (Invitrogen) and 100 μg/mL streptomycin (Invitrogen).

Mouse bone marrow-derived dendritic cells (BMDCs) were prepared as described previously [14]. In brief, bone marrow cells were isolated from C57BL/6 mouse femur and residual tissue was removed using a 70-μm nylon cell strainer (BD Bioscience, Franklin Lakes, NJ, USA). Cells were plated in 100-mm dishes (1×10^7 cells/dish) and cultured in RPMI-1640 medium supplemented with 10% FBS, 100 μg/mL streptomycin, 100 IU/mL penicillin, 10 mM HEPES, 50 μM β-mercaptoethanol, and 20 ng/mL GM-CSF. Medium was replaced every 2 days. On day 6 after plating, aggregates of immature DCs were pooled and used in subsequent experiments. The percentage of CD11c + cells in these preparations was >85%, as verified using the Cytomics™ FC500 flow cytometer (Beckman Coulter, Miami, FL).

C57BL/6 female mice 6–8 weeks old and weighing 18–22 g were obtained from Vital River LCM (Beijing, China) and housed in a specific pathogen-free, light-cycled and temperature-controlled facility. All animal experiments were approved by the Institutional Animal Care and Ethics Committee of Sichuan University.

2.4. Synthesis of PEI-2k-stearic acid (PSA)

Since Trp2 is relatively hydrophobic, we conjugated branched PEI-2k to stearic acid in order to form the amphiphilic PSA molecule. Briefly, stearic acid (71.2 mg) was dissolved in 10 mL DMSO, and then EDCI (57.5 mg) and NHS (34.5 mg) were added. The mixture was stirred at room temperature for 2 h to allow sufficient activation of the carboxyl group on the stearic acid. 752.1 mg of PEI-2k in DMSO was added to the mixture. After the reaction was allowed to stand for 24 h, residual stearic acid was extracted with ethylacetate and the PSA was further purified by dialysis against distilled water for 2 days using a membrane with an 8–14 kDa molecular weight cut-off. The dialysate was freeze-dried to yield the final PSA. The identity of the conjugate was verified by ^{1}H NMR in 4D-CH_3OH (Sigma-Aldrich) using a Varian Unity Inova 400 spectrometer at 400 MHz, as well as by FTIR spectroscopy using a Vector 22 spectrometer (Bruker, Ettlingen, Germany).

2.5. Preparation of Trp2-loaded PSA micelles and analysis of encapsulation efficiency

Trp2-loaded PSA micelles were self-assembled by dialysis. Briefly, various amounts of PSA (1.0–8.0 mg) were dissolved in methanol (2 mL), and then added to a solution of Trp2 peptide (1.0 mg) in 100 μL DMSO. After incubating the mixture for 30 min at 55 °C, the solvents were removed by dialysis against distilled water. The dialysate was freeze-dried in the presence of 5% glucose as the cryoprotectant, and rehydrated with sterile distilled water to yield Trp2-loaded PSA micelles. Levels of encapsulated Trp2 were determined using the reverse-phase HPLC gradient method (Agilent Technologies 1260 Infinity, Hewlett Packard, Wilmington, NC, USA).

2.6. Analysis of size and zeta potential of PSA-loaded micelles

Trp2-loaded PSA micelles prepared using the various amounts of PSA and Trp2 mentioned above were analyzed by dynamic light scattering to determine average diameter and by electrophoretic light scattering to determine zeta potential. Measurements were collected on a photon correlation spectrometer equipped with a 50-mV laser (Zetasizer Nano ZS90, Malvern Instruments, UK).

Please cite this article as: Q. Zeng, et al., Cationic micelle delivery of Trp2 peptide for efficient lymphatic draining and enhanced cytotoxic T-lymphocyte responses, J. Control. Release (2014), http://dx.doi.org/10.1016/j.jconrel.2014.12.024