Carbohydrate Polymers 92 (2013) 1586–1595

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Mannose-poly(ethylene glycol)-linked SPION targeted to antigen presenting cells for magnetic resonance imaging on lymph node

Muthunarayanan Muthiah[a,b,1], Hieu Vu-Quang[a,b,1], You-Kyoung Kim[c], Joon Haeng Rhee[b], Sang Hyeon Kang[d], Soo Youn Jun[d], Yun-Jaie Choi[c], Yong Yeon Jeong[e], Chong-Su Cho[c,**], In-Kyu Park[a,b,*]

[a] Department of Biomedical Sciences and Center for Biomedical Human Resources (BK-21 project), Chonnam National University Medical School, Gwangju 501-757, South Korea
[b] Clinical Vaccine R&D Center, Chonnam National University Hwasun Hospital, Jeonnam 519-763, South Korea
[c] Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, South Korea
[d] iNtRON Biotechnology, Inc., Seongnam 462-120, South Korea
[e] Department of Radiology, Chonnam National University Hwasun Hospital, Jeonnam 519-763, South Korea

ARTICLE INFO

Article history:
Received 27 September 2012
Received in revised form 3 November 2012
Accepted 4 November 2012
Available online 13 November 2012

Keywords:
Mannose
SPION
Mannose receptor
Antigen-presenting cells
Lymph node
MR imaging

ABSTRACT

The aim of this study is to prepare biocompatible and targetable nanoparticles in lymph nodes (LNs) for lymph node-specific magnetic resonance (MR) imaging. Mannan-coated superparamagnetic iron oxide nanoparticles (SPIONs) (mannan-SPION), carboxylic mannan-coated SPION (CM-SPION), and β-glucan-coated SPION (Glucan-SPION) have been developed to target antigen-presenting cells (APCs), for lymph node detection by MR imaging. In this study, mannose-polyethylene glycol (PEG) was prepared by conjugating D-mannopyranosylphenyl isothiocyanate and amine-PEG-carboxyl. The 3-aminopropyltriethoxysilane (APTES)-activated SPION and the mannose-PEG were cross-linked to produce mannose-PEG-linked SPION (Mannose-PEG-SPION). Mannose-PEG-SPION carrying mannose on the surface were assumed efficient at targeting APCs through the specific interactions of the mannose tethered on the Mannose-PEG-SPION and the mannose receptors on the antigen presenting cells. The hydrophilic PEG corona layer in the Mannose-PEG-SPION could be prevented from aggregation during the systemic circulation with accompanying enhanced specificity and minimized systemic toxicity. The accumulation of SPION in the lymph nodes led to increased negative enhancement in the MR images. In the *in vivo* study, rats were injected intravenously with Mannose-PEG-SPION and PEG-SPION, as a control and then tracked by MR imaging after 1 h, 2 h, 3 h, and 24 h. MR imaging on lymph nodes clearly revealed the preferential uptake of Mannose-PEG-SPION in immune cell-rich lymph nodes. The predominant accumulation of Mannose-PEG-SPION in the lymph nodes was also confirmed by Prussian blue staining. Based on these results, Mannose-PEG-SPION shows great potential for lymph node-specific MR imaging.

1. Introduction

Targeted delivery to the site of action has clear therapeutic advantages because it maximizes therapeutic efficiency and minimizes systemic toxicity (Vyas, Singh, & Sihorkar, 2001). Other advantages of targeted delivery are simplified administration and reduced amount of therapeutic molecules required for successful therapy. However, the main advantage is that the local concentration of the therapeutic molecules is elevated, thus protecting the normal cells in cases of cancer and providing more safety and biocompatibility. Considering the immunogenicity of antibodies, carbohydrates were explored for targeted drug or gene delivery and imaging. Specific interactions of carbohydrates with endogenous lectins showed a large number of carbohydrate-binding proteins expressed on mammalian cell surfaces (Yamazaki et al., 2000). Increased expression of lectins was confirmed in malignant cells that are believed to be involved in cancer metastasis, and thus are potential targets for the delivery of drug, gene, or imaging agents to malignant cells (Gabius, 2004). Lectins were investigated as potential targeting moieties for cell surface carbohydrates. Macrophages express carbohydrate receptors, such as the macrophage–mannose receptor, to recognize invaded pathogens. Mannose receptors were expressed abundantly in the immune cells, such as Kupffer cells in

* Corresponding author at: Department of Biomedical Sciences, Chonnam National University Medical School, 5, Hak-1-dong, Gwangju 501-746, South Korea. Tel.: +82 61 379 8481; fax: +82 61 379 8455.
** Corresponding author. Tel.: +82 2 880 4868; fax: +82 2 875 2494.
E-mail addresses: chocs@snu.ac.kr (C.-S. Cho), pik96@chonnam.ac.kr, pik96d@gmail.com (I.-K. Park).
[1] These two authors have contributed to this work equally.

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.11.011

the liver, sinusoidal lining cells in the spleen, alveolar macrophages, macrophages in the lymph nodes, and immune cells near the tumor microenvironment. The inclusion of specific ligands on the surface of nanoparticles was shown to enhance significantly the rates and extent of uptake by immune cells such as APCs (Jiang et al., 2009; Kim, Jin, Kim, Cho, & Cho, 2006). Macrophages accumulate at pathological sites, including infection, tumors, atherosclerotic plaques, and arthritic joints and are an important target for drug delivery, gene delivery, and tissue-specific imaging. In our previous study, we demonstrated that mannan-coated SPION (mannan-SPION) was specific to immune cells in LN because of mannose receptor-mediated endocytosis, facilitation of preferential uptake in APCs, faster acquisition, and an enhanced contrast of the MR imaging in target tissues, as compared with Dextran-coated SPION (Dex-SPION) and PVA-coated SPION (PVA-SPION) (Hieu et al., 2011). It was also previously reported by our group that SPION coated with β-glucan could target APCs because glucan was reported to elicit immune responses through the activation of macrophages by an immune cell-specific (1, 3)-β-D-glucan receptor or dectin-1 receptor. β-Glucan-coated SPION was internalized by the immune cells residing in the metastatic liver, which aided in discrimination between metastasized tumor regions and normal hepatic parenchymal tissue. β-Glucan also induces the immune system, which aids in anti-tumor activity (Vu-Quang, Muthiah, Lee, et al., 2012). However, the mannose component of the *Candida albicans* cell wall was identified as a stimulus for tumor necrosis factor alpha (TNFa) secretion (Ataoglu, Dogan, Mustafa, & Akarsu, 2000). It is known that TNFa is among the cytokines that have endogenous pyrogenic activity. The pyrogenic effects of systemically injected yeast mannoses that originated from *C. albicans* and *Saccharomyces cerevisiae* were previously reported in rabbits. We hypothesized that the conjugation of mannose on the surface of iron oxide nanoparticles with PEG linker might reduce this undesired toxic response. This hypothesis is based on the fact that PEGylation has many advantages, such as prolonged residence in the body, decreased degradation by metabolic enzymes, and reduction or elimination of protein immunogenicity (Yuan, Fabregat, Yoshimoto, & Nagasaki, 2011). It was demonstrated that PEGylation is a useful strategy to minimize the biofouling and aggregation of SPION in physiological conditions because of its hydrophilicity and steric repulsion of PEG chains (Islam et al., 2010; Liu et al., 2011).

In this work, we synthesized mannose-PEG-linked SPION (Mannose-PEG-SPION) for targeting to APCs through the specific interactions of mannose on the SPION with mannose receptor on APCs in the lymph node, which can be tracked by MR imaging. The presence of Mannose-PEG-SPION in lymph nodes was also confirmed by histological analysis.

2. Materials and methods

NH2-PEG-COOH (Mw 2 kDa) was purchased from NOF Corporation, USA. D-Mannopyranosylphenyl isothiocyanate, ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O > 97\%$), and ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O > 99\%$) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of analytical reagent grade and were used without further purification.

2.1. Synthesis and characterization of mannose-PEG-COOH and Mannose-PEG-SPION

Mannose-PEG-COOH was prepared without reagents from the direct conjugation of NH_2-PEG-COOH (2 kDa) and D-mannopyranosylphenyl isothiocyanate. Both chemicals were dissolved in dimethylsulfoxide (DMSO) and agitated at 25 °C for 24 h. The SPION was synthesized by a modified co-precipitation method, in accordance with a previous study (Yoo et al., 2008). Following the addition of NH_4OH, SPION was prepared by an alkaline co-precipitation of $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 4H_2O$ with a Fe(III)/Fe(II) feed ratio of 3:1 in deoxygenated water. The black precipitate was washed several times, and the final pH was adjusted from 10 to 7. The solution was allowed to sediment under a magnetic field, and the supernatant was discarded. The black sediment was mixed with 2 M HNO_3 and 0.35 M $Fe(NO_3)_3$. The suspension was dialyzed for 2 days against 0.01 M HNO_3 and stored at 4 °C. The prepared SPION was mixed with 3-aminopropyltriethoxysilane (APTES) and dissolved in ethanol under a nitrogen atmosphere for 24 h to produce APTES-activated SPION (APTES-SPION). APTES-SPION were then conjugated to an excess amount of mannose-PEG-COOH (10 times by weight) with an EDC/NHS coupling agent at 25 °C for 24 h. The amount of PEG conjugated to SPION was optimized by thermogravimetric analysis. The synthesized mannose-PEG-COOH was analyzed by ^1H nuclear magnetic resonance spectroscopy (^1H NMR, Avance_600, Bruker, Germany). The Fourier transform infrared (FT-IR) spectra were measured using a Nicolet Magna 550 series II (Midac, Atlanta, GA, USA). The particle sizes of the Mannose-PEG-SPION were assessed using an electrophoretic light-scattering spectrophotometer (ELS 8000, Otsuka Electronics, Osaka, Japan), with 90° and 20° scattering angles at 25 °C. An electrophoretic mobility measurement was performed using the same setting equipped with a platinum electrode. The morphology of Mannose-PEG-SPION was also obtained by using a JEOL JEM-1010 transmission electron microscope (TEM).

2.2. Primary peritoneal macrophage isolation

Peritoneal macrophages were isolated as reported in our previous study (Vu-Quang, Muthiah, Kim, et al., 2012). In brief, 3 ml of Brewer thioglycollate medium (Fluka, Mumbai, India) was injected first into the peritoneal cavity of inbred Balb/c mice (7 weeks, male) (Orient Bio Inc., Seongnam-si, South Korea). 3 days after injection of Brewer thioglycollate medium, ice cold RPMI 1640 (Thermo Scientific, Logan, UT) was injected into the peritoneal cavity, in order to suspend the enriched peritoneal macrophages. The fluid was withdrawn, washed with PBS, and centrifuged three times at 1000 r.p.m. for 10 min. Macrophages were allowed to adhere to the cell culture dish in serum-free RPMI 1640 for 2 h at 37 °C and 5% CO_2. The cells were washed with cold PBS three times and the adherent cells harvested using a cell scraper.

2.3. Cytotoxicity of Mannose-PEG-SPION

Isolated peritoneal macrophages were seeded in 96 well plates at a density of 2×10^4/well in 100 μL of RPMI 1640 growth medium and incubated overnight at 37 °C in 5% CO_2. The cells were treated with Mannose-PEG-SPION, PEG-linked SPION (PEG-SPION), and SPION at various concentrations (50–500 μg Fe/ml) for 24 h. The cells were then incubated with 100 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution for 4 h to allow the formation of formazan crystals by mitochondrial dehydrogenases. The medium was removed, and 100 μL of DMSO was added to dissolve the formazan crystals. The optical density of the solution was measured at a wavelength of 570 nm, using a Spectra Max 190 spectrophotometer (Molecular Devices, Sunnyvale, CA).

2.4. Systemic toxicity of Mannose-PEG-SPION

A toxicological comparison was performed between Mannose-PEG-SPION and mannan-SPION, using the conventional single-dose intravenous toxicity test. The test was performed at the Korea Institute of Toxicology, Korea Research Institute of Chemical