International Journal of Pharmaceutics 373 (2009) 165–173

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Hyaluronic acid coated poly(butyl cyanoacrylate) nanoparticles as anticancer drug carriers

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ARTICLE INFO

Article history:
Received 15 October 2008
Received in revised form 17 January 2009
Accepted 12 February 2009
Available online 24 February 2009

Keywords:
Hyaluronic acid
Poly(butyl cyanoacrylate)
Nanoparticles
Paclitaxel
Drug delivery
Antitumor effect

ABSTRACT

The hyaluronic acid (HA) coated poly(butyl cyanoacrylate) (PBCA) nanoparticles were synthesized through radical polymerization of butyl cyanoarylate (BCA) initiated by cerium ions in the presence of HA. The chemical coupling between HA and PBCA was demonstrated by FTIR, ^{1}H NMR and X-ray diffraction. The sizes of the nanoparticles with different HA/BCA ratios were 291–325 nm at cerium concentration of 0.8 mmol/L and HA molecular weight of 18,000 Da. Paclitaxel (PTX), a model anticancer drug, was encapsulated in negatively charged nanoparticles with a maximal encapsulation efficiency of 90%. In vitro release demonstrated that HA modification could effectively reduce the initial burst release in the first 10 h and provide a sustained release in the subsequent 188 h. As evidenced by the hemolysis assay and MTT assay, HA coating could significantly reduce the cytotoxicity. Cellular uptake indicated that uptake of HA-PBCA nanoparticles by Sarcoma-180 (S-180) cells was 9.5-fold higher than that of PBCA nanoparticles. PTX-loaded HA-PBCA nanoparticles were more potent in tumor growth suppression than PTX-loaded PBCA nanoparticles or PTX injection following intravenous administration to S-180 tumor bearing mice. Therefore, the HA-PBCA nanoparticles could be an effective and safe vehicle for systemic administration of hydrophobic anticancer drugs.

1. Introduction

Nanoparticles have been studied as vehicles for antitumor drugs due to their small size, prolonged in vivo circulation and sustained drug release. It has been demonstrated that the surface property of nanoparticles is the key factor in modulating their biodistribution parameters (Lourenco et al., 1996; Peracchia et al., 1998). Nanoparticles with more hydrophobic surfaces will be preferentially taken up by the liver, and subsequently by the spleen and lung (Brigger et al., 2002). Plasma proteins and complement activation play a major role in the recognition of the particles by macrophages of the mononuclear phagocytes system (MPS) and in their rapid clearance from the bloodstream. In comparison, hydrophilic polymers can create a cloud of chains at the particle surface that will repel plasma proteins. Thus, the surfaces of nanoparticles with hydrophilic modification are designed in order to reduce the opsonization.

Poly(alkylcyanoacrylate) as drug carriers have been investigated extensively for nearly 30 years due to their low toxicity and biodegradability. Poly(alkylcyanoacrylate) nanoparticles were firstly developed through anionic emulsion polymerization by Couvreur et al. (1979). However, the nanoparticles obtained were rapidly cleared from the bloodstream by macrophage uptake. To prevent this uptake it has been suggested to modify the surface with more hydrophilic polymers. The most popular approach is grafting poly(ethylene glycol) (PEG) chains at the particle surface. The tissue distribution of PEG-coated poly(alkylcyanoacrylate) nanoparticles mainly depend on the different microvascular permeability between healthy tissues and tumors as well as their long circulating properties (Bazile et al., 1995; Chauvierre et al., 2003a). Another approach is coating nanoparticles with polysaccharides that have specific receptors in certain cells or tissues, thus achieving active targeting (Lemarchand et al., 2006). Some of the polysaccharides also have biological activity. For example, heparin has been used to prepare anticancer carrier due to its antiproliferative effect on tumor and endothelial cells (Park et al., 2006). Therefore, polysaccharide coated nanoparticles could be a new tendency in drug delivery systems. Chauvierre et al. (2003a) prepared polysaccharide modified poly(isobutyl cyanoacrylate) nanoparticles that preserved the biologic activity of the polysaccharide. The properties of nanoparticles depended on the polysaccharide. However, hyaluronic acid (HA), chitosan, and pectin formed microparticles with diameters from 30 to 59 μm which limited their application in drug delivery system due to the large particle size.

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doi:10.1016/j.ijpharm.2009.02.012

HA is a linear, negatively charged polysaccharide, consisting of two alternating units of D-glucuronic acid and N-acetyl-D-glucosamine. As a major component of extracellular matrix in connective tissues, vitreous, and synovial fluids, it plays a critical role in cell growth, differentiation, migration, wound healing, and even cancer metastasis (Entwistle et al., 1996). HA has been used to prepare microspheres as gene delivery vehicle (Yun et al., 2004). Besides, HA-binding receptors CD44 (Day and Prestwich, 2002) and RHAMM (Yang et al., 1993) are over-expressed in various tumors. The HA level is elevated in cancer cells (Toole et al., 2002), forming a less dense matrix and leading to the enhanced cell motility and invasive ability into healthy tissues. Because of its high tumor specificity and biocompatibility, HA could be potentially used for the design of tumor-targeting drug delivery vehicles for anticancer drugs such as paclitaxel (PTX).

In the present study, HA coated poly(butyl cyanoacrylate) (PBCA) nanoparticles were prepared as an anticancer drug delivery vehicle through radical emulsion polymerization of n-butyl cyanoacrylate (BCA) monomers initiated by cerium ions in the presence of HA. The nanoparticles were characterized by FTIR, ^{1}H NMR, X-ray diffraction, photon correlation spectroscopy (PCS), zeta potential analysis, and transmission electron microscopy (TEM). With PTX as a model anticancer drug, the encapsulation efficiencies of the nanoparticles and in vitro drug release behavior were investigated. The biocompatibility of the nanoparticles was estimated in terms of hemolysis and MTT assay. The Sarcoma-180 (S-180) cellular uptake of PBCA and HA coated PBCA nanoparticles were determined. Finally, the anticancer efficiency of PTX-loaded nanoparticles was investigated in S-180 bearing mice.

2. Materials and methods

2.1. Materials

HA (MW18, 100, 500 and 1000 kDa) were obtained from Zhenjiang Dong Yuan Biotech Company Limited (Jiangsu, China). BCA monomers were obtained from Suncon Medical Adhesive Company (Beijing, China). Acetonitrile (Merck, Germany) was of HPLC grade. All the other reagents were of analytical grade.

Male KM mice weighing 18–22 g were provided by the Animal Care Center, Fudan University. Male New Zealand rabbits weighing 2.0–2.5 kg were provided by the Animal Care Center, Second Military Medical University.

S-180 cells and HEK-293 cells were purchased from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). S-180 cells were cultured in RPMI-1640 medium (Gibco, USA), supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 2 mmol/L glutamine, and 0.1 mg/mL of both penicillin and streptomycin. HEK-293 cells were cultured in Dulbecco's modified Eagle medium (Gibco, USA). Cells were cultured in a humidified atmosphere of 5% CO_2 at 37 °C.

Animal study protocol was reviewed and approved by the Institutional Animal Care and Use Committee, Fudan University, China.

2.2. Preparation and characterization of HA-PBCA nanoparticles

2.2.1. Preparation of HA-PBCA nanoparticles

HA-PBCA nanoparticles were prepared by radical polymerization of HA and BCA monomers. Briefly, HA was dissolved in 9 mL of nitric acid (0.2 mol/L) under gentle stirring and nitrogen gas bubbling. Ten minutes later, a solution of cerium ammonium nitrate was added, followed by addition of BCA monomers mixed with 1 mL of nitric acid under vigorous agitation. The pH of the polymerization system was maintained at around 0.7. The reaction was terminated after 3 h. The obtained nanoparticle suspensions were dialyzed against water for 48 h (molecular weight cut-off of 3500 Da). To study the influence of the reaction conditions on the characteristics of nanoparticles, various HA/BCA ratios (1:1–1:6 (w/w)), cerium ammonium nitrate concentrations (0.4–1.4 mmol/L), and HA molecular weight were used.

Unmodified nanoparticles were prepared as follows. Briefly, 50 μL of BCA monomers were dispersed in 5 mL of pH 2.5 HCl solution containing 0.5% poloxamer 188 as stabilizer and were allowed to polymerize spontaneously for 4 h under vigorous stirring. The obtained nanoparticle suspensions were dialyzed against water for 48 h (molecular weight cut-off of 3500 Da).

2.2.2. Characterization of HA-PBCA nanoparticles

Infrared spectra of HA and lyophilized HA-PBCA nanoparticles were obtained using a Thermo Nicolet Nexus 470 FTIR spectrometer (USA).

The lyophilized HA-PBCA nanoparticles were dissolved in a mixed solvent of 0.5 mL of DMSO-d_6 (99.9 atom% deuterium, Cambridge Isotope Laboratories, Inc.) and 100 μL of trifluoroacetic acid-D (99.5 atom% deuterium, Cambridge Isotope Laboratories, Inc.). The internal reference was tetramethylsilane (TMS). ^{1}H NMR spectrum was recorded with a Bruker AVANCE DMX 500 spectrometer (Germany).

X-ray diffraction spectra were obtained using a D/max-γB multicrystal diffraction meter (Rigaku, Japan) with CuKα radiation in the range of 2.5–50°(2θ) at 40 kV and 60 mA.

2.3. Particle size measurement

The particle size of the nanoparticles was measured by photon correlation spectroscopy with a Zeta Potential/Particle Sizer (Nicomp^{TM}380 ZLS, USA). All measurements were done at a wavelength of 635 nm at 23 °C with an angle detection of 90°. The particle size was expressed by mean effective diameter.

2.4. Surface charge

Zeta potential of the nanoparticles was measured on PCS (Nicomp^{TM}380 ZLS, USA). Each sample was measured three times at 23 °C.

2.5. Morphology

Nanoparticle suspensions were mounted on a Formvar-coated copper grid and subjected to negative staining with sodium phosphotungstate solution (0.2%, w/v). After 3 min of incubation at room temperature, the grid was air-dried and the morphology of the nanoparticles was visualized using a TEM (H-600A, Hitachi, Japan).

2.6. Preparation and characterization of PTX-loaded HA-PBCA nanoparticles

2.6.1. Preparation of PTX-loaded HA-PBCA nanoparticles

PTX in ethanol was slowly added to HA-PBCA nanoparticle suspensions under stirring. The resulting suspensions were sonicated for 240 s at 60 W output, dialyzed against water to remove residual ethanol and filtered through 0.80 μm membrane to remove PTX precipitates.

2.6.2. Encapsulation efficiency

Silica gel column (1.5 cm × 15 cm) was equilibrated with saline, and the PTX-loaded nanoparticle suspensions were loaded. The column was eluted with saline at a flow rate of 1.0 mL/min in order to separate nanoparticles from free PTX solubilized in nanoparticle suspensions. Different gradient of ethanol solutions were then used