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Amphiphilic glycopolymer nanoparticles as vehicles for nasal delivery of peptides and proteins

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ABSTRACT

Nasal drug delivery system has been a very promising route for delivery of proteins and peptides for the reason that it can avoid degradation in gastrointestinal tract and metabolism by liver enzymes. However, the bioavailability of proteins and peptides is still low due to the rapid clearance of mucociliary. Here, to prolong the residence time of drugs and improve their absorption, we prepared amphiphilic glycopolymer poly(2-lactobionamidoethyl methacrylate-random-3-acrylamidophenylboronic acid) (p(LAMA-*r*-AAPBA), and the glycopolymer could assemble into the nanoparticles with narrow size distribution. Insulin, as a model drug, was efficiently encapsulated within the nanoparticles, and loading capacity was up to 12%. In vitro study revealed that the insulin release could be controlled by modifying the composition of glycopolymers. Cell viability showed that p(LAMA-*r*-AAPBA) nanoparticles had good cytocompatibility. Moreover, the mechanism of nanoparticle internalization into Calu-3 cells was a combination mechanism of clathrin-mediated endocytosis and lipid raft/caveolae-mediated endocytosis. Importantly, there was a significant decrease in the blood glucose levels after the nasal administration of p(LAMA-*r*-AAPBA) nanoparticles to diabetic rats. Therefore, p(LAMA-*r*-AAPBA) glycopolymers have a potential application as a nasal delivery systems for proteins and peptides.

1. Introduction

In recent years, advances in biotechnology have led to the development of various significant peptides and proteins which play an important role in diagnosis and medical treatment for various diseases. Therapeutic peptides and proteins are typically administered by injection due to their fragile nature. Thus, chronic treatment brings incontinences and pains to the patient. One possible way to overcome these problems is to develop non-invasive administrations, such as nasal, gastrointestinal and ophthalmic administrations. Among these administrations, nasal administration has attracted great interest as an alternative route for the systemic delivery of protein drugs, such as insulin (Arora et al., 2002), and it can avoid degradation of drug resulting from acidic or enzymatic degradation in gastrointestinal tract and hepatic first pass metabolism. Several potential advantages contribute to the attainment of adequate bioavailability of proteins drugs comparable to the other administration, including the large surface area of nasal mucosa, a porous endothelial membrane, and a highly vascularized epithelium.

Although nasal delivery is non-invasive, painless, and can permit self-administration (Mygind and Dahl, 1998), there are still some factors affecting the permeability of drugs through the nasal mucosa, such as the normal defense mechanisms like mucocillary clearance, ciliary beat frequency and inflammation, and enzymatic barrier (Duan and Mao, 2010). To conquer these barriers, we developed a new type of phenylboronic acid-functionalized glycopolymers. The glycopolymer nanoparticles are expected to prolong the residence time of the formulations in the nasal cavity and protect the encapsulated peptides and proteins from enzyme degradation during transporting them across mucosal epithelial cells.

The boronic acid and its derivatives are known to possess the ability to reversibly interact with diols, sugars, and glycoproteins, and transport saccharide across lipid bilayers (Cambre and Sumerlin, 2011; Norrild, 2012; Westmark and Smith, 1994). Since all cell membranes virtually include glycoproteins and/or glycolipids, which facilitates compounds containing boronic acid groups to bind to cell surfaces (Vandenburg et al., 2000). It has been confirmed in our previous work that the copolymers with phenylboronic acid pendant groups could interact with mucin (Zhang et al., 2012) which was the major component of the mucus that

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coated the cells lining the surfaces of the respiratory, digestive, and urogenital tracts. Moreover, other researches revealed that phenylboronic acids were potent inhibitors of serine proteases such as trypsin, chymotrypsin, elastase, and leucine aminopeptidase (Smoum et al., 2003). The application of phenylboronic acid and their derivatives is limited due to their cytotoxicity (Achanta et al., 2006; Qureshi et al., 2001). But our previous study confirmed that the introduction of the carbohydrates to polymers based on phenylboronic acid, which have abundant hydroxyl groups, could improve the hydrophilicity and biocompatibility of phenylboronic acid (Jin et al., 2009; Wang et al., 2012).

In the present work, we propose to design new drug delivery vehicles on the basis of poly (2-lactobionamidoethyl methacrylate-*r*-3-acrylamidophenylboronic acid). The obtained glycopolymers had the amphiphilicity with random composition of 2-lactobionamidoethyl methacrylate (LAMA) and 3-acrylamidophenylboronic acid (AAPBA). It is anticipated that the glycopolymers could increase the transport of insulin through the respiratory epithelia. The cytotoxicity and mucoadhesion of the phenylboronic acid-functioned glycopolymers synthesized were evaluated, and the cell uptake mechanism of nanoparticles was investigated. The hypoglycemic effect on diabetic rats after that nasal administration of insulin-loaded nanoparticles was also conducted.

2. 2. Materials and methods

2.1. Materials

3-Aminophenylboronic acid monohydrate was purchased from Nanjing Kangmanlin Chemical Industry Co. Ltd. (Nanjing, China) without further purification before use. (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) (MTT), 1,1'-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI), Wortmannin and Fluorescein isothiocyanate (FITC) were purchased from Beyotime Institute of Biotechnology (Nantong, China). Methyl-β-cyclydextrin was obtained from Aladdin-regeant (Shanghai, China) Co., Ltd. Mucin type III (∼1% sialic acid), nystatin, amiloride and chlorpromazine were purchased from the Sigma-Aldrich (USA), and used without further purification. Methacryloyl chloride and acryloyl chloride was directly prepared by refluxing thionyl chloride with methacrylic acid and acrylic acid, respectively, for 8 h at 90 °C and freshly distilled before use. Ethanolamine hydrochloride was obtained from dropping hydrochloric acid to the maxed solution of 2-aminoethanol and water for 2 h at 90 °C and then rose the temperature to 105–110 °C for a further reaction of 1 h. 2-Aminoethyl methacrylate was synthesized according to the method reported previously. Lactobionolactone was obtained from dehydration of commercial lactobionic acid.

2.2. Synthesis of 2-lactobionamidoethyl methacrylate (LAMA)

2-Lactobionamidoethyl methacrylate (LAMA) was prepared according to the method reported previously (Narain and Armes, 2003). Lactobionolactone (10.0 g, 29.4 mmol) was dissolved in methanol at 40 °C and then cooled to room temperature. Then 2-aminoethyl methacrylate hydrochloride (10.0 g, 60.4 mmol), triethylamine (10.0 mL) and hydroquinone (0.25 g) were added to the above solution. The mixture was stirred for 5 h, concentrated under vacuum and precipitated into isopropanol. The obtained solid was filtered, washed with isopropanol and dried under vacuum.

2.3. Synthesis of 3-acrylamidophenylboronic acid (AAPBA)

3-Acrylamidophenylboronic acid (AAPBA) was synthesized according to the method described by Lee et al. (2004) with minor changes. Briefly, 3-aminophenylboronic acid monohydrate (5.0 g, 32.2 mmol) was dissolved in sodium hydroxide solution (40 mL, 129.0 mmol) and cooled in an ice bath. Freshly distilled acryloyl chloride (3.2 mL, 40.0 mmol) was dropped to 3-aminophenylboronic acid monohydrate solution, over a period of 1 h. After cooling to room temperature, the reaction mixture was stirred for further 2 h. The pH of the mixture was adjusted to pH 8 using 0.1 M HCl. The resulting precipitate was filtered and washed with cold water. The precipitate was dissolved in distilled water after heating to 80 °C, and filtered. The filtrate was left to stand overnight in room temperature, and the resulting crystals were filtered, and dried under a vacuum.

2.4. Synthesis of glycopolymer poly(2-lactobionamidoethyl methacrylate-r-3-acrylamidophenylboronic acid) (p(LAMA-r-AAPBA))

Copolymer was synthesized by a conventional radical polymerization method by using azobisisobutyronitrile (AIBN) as an initiator (Scheme 1). Briefly, AAPBA and LAMA were added to dimethyl sulfoxide (DMSO) in a three-necked, round flask equipped with reflux condenser. After purging N_2 for 30 min, mercaptoethylamine was added as chain transfer agent. Copolymerization was initiated by AIBN (8% molar of the total monomers) at 70 °C. The copolymerization was continued for 5 h and the precipitated glycopolymer was filtered and dried under a vacuum. By changing the feed molar ratio of LAMA to AAPBA (2:1, 1:1 and 1:2), three distinct glycopolymers were obtained. For simplicity, the glycopolymers were marked as p(LAMAa-*r*-AAPBAb) in which letters "a" and "b" represented the feed molar ratio of the copolymerization reaction (Table 1).

2.5. Characterization of the glycopolymer p(LAMA-r-AAPBA)

Proton nuclear magnetic resonance (^{1}H NMR) spectra of LAMA, AAPBA and p(LAMA-*r*-AAPBA) were recorded at room temperature using a Varian Unity-plus 400 NMR spectrometer. FT-IR spectrum of p(LAMA-*r*-AAPBA) was recorded on a Fourier Tranform Infrared Spectrometer (Bio-Rad FTS-6000) with a KBr tablet containing the powders of the sample at a resolution of 8 cm^{-1}.

2.6. Preparation of the nanoparticles

Nanoparticles were prepared via the self-assembly method (Bes et al., 2003; Peroche et al., 2005). Briefly, 2 mg glycopolymer p(LAMA-*r*-AAPBA) was dissolved in 2 mL mixed solvent of DMSO and H_2O (1:1, v/v) and 18 mL distilled water was added to the solution under stirring. The procedure yielded an opalescent suspension and then the solution was incubated overnight at room temperature with stirring. The resulting nanoparticles were transferred to a dialysis tube (MWCO 3500) and dialyzed in water for 24 h. Nanoparticles were separated by centrifuging at 12,000 rpm for 20 min, and then freeze-dried.

The insulin-loaded nanoparticles were prepared by dropping 2 mL of 1 mg/mL p(LAMA-*r*-AAPBA) DMSO/H_2O (v:v, 1:1) solution into 18 mL insulin solution (500 μg/mL) under stirring, and then with continuously stirring for 24 h. After dialysis, the insulin-loaded nanoparticles were separated by centrifuging at 12,000 rpm for 20 min at 4 °C, washed three times with water and then freeze-dried.

2.7. Characterization of nanoparticles

The size and zeta potential of the nanoparticles was determined by dynamic light scattering analyzer (Malvern, Nano ZS90/