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β-Cyclodextrin grafting hyperbranched polyglycerols as carriers for nasal insulin delivery

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ABSTRACT

We prepared a β-CD functionalized hyperbranched polyglycerol (HPG) with the purpose of enhancing the nasal transport of insulin in rats. Insulin-loaded HPG-*g*-CD nanoparticles (NPs) were prepared and the size of the NPs ranged from 198 to 340 nm with a positive charge. The NPs exhibited a great capacity of associating insulin, reaching the efficiency as high as 88.21%. *In vitro* release showed that the release rate of insulin was much faster under acidic condition than physiological condition. *In vitro* cytotoxicity against Caco-2 cells showed that HPG-*g*-CDs had good biocompatibility. The *in vivo* evaluation in rats demonstrated that insulin-loaded HPG-*g*-CD NPs had the ability to significantly decrease the blood glucose concentrations. Furthermore, the capability of HPG-*g*-CD NPs to cross the nasal mucosal epithelia was proved by confocal laser scanning microscopy (CLSM). Consequently, the results suggest that the HPG-*g*-CD NPs are promising carriers for nasal insulin delivery.

1. Introduction

Peptide and protein drugs, including insulin, are usually used as parenteral formulations, but this method of administration is sometimes associated with tissue invasion and infection. Oral delivery of insulin can replace daily injections to diabetic patients. However, poses unique problem of stability and susceptibility to proteolysis, which reduce their bioavailability (Shelma, Paul, & Sharma, 2010). Recently, researches have been focused on the nasal mucosa as an alternative route to the oral and parenteral routes, since it has many advantages. These include a large absorptive surface area and the high vascularity of the nasal mucosa, where drugs absorbed from the nasal cavity pass directly into systemic circulation, thereby avoiding first-pass liver metabolism (Chein & Chang, 1987).

However, the bioavailability of intranasal administered peptide and protein drugs may be low due to their high molecular weight and hydrophilicity (Merkus, Verhoef, Romeijn, & Schipper, 1991). In addition, the normal physiology of the nasal cavity presents several barriers to peptide and protein drugs absorption, including the physical removal by mucociliary clearance mechanism, enzymatic degradation and low permeability of the nasal epithelium (Wu, Wei, Wang, Su, & Ma, 2007).

Many strategies have been explored to improve the absorption of these drugs through the nasal mucosa, including the use of chemical penetration enhancers and proteolytic enzyme inhibitors, and designing suitable dosage formulations. Among these approaches, the use of absorption enhancers has been proven to be effective (Wang et al., 2002). Unfortunately, most of the traditional absorption enhancers, such as surfactants and bile salts, have limited clinical use because of the irreversible damage to the nasal mucosa when used at effective concentrations, particularly under long-term exposure (Khafagy, Morishita, Onuki, & Takayama, 2007).

Cyclodextrin (CD) can form non-covalent inclusion complexes with a large variety of drugs/proteins. Complexation represents a unique and effective strategy for improving protein therapy by stabilizing the drugs against aggregation, thermal denaturation and degradation (Sajeesh & Sharma, 2006). Moreover, CDs are believed to enhance nasal absorption of peptides by opening tight junctions and/or solubilizing membrane components (Marttin, Verhoef, & Merkus, 1998), which perturbs membrane integrity in a rather nonspecific manner. It is inevitable that varying extents of assault would occur to the mucosal tissue in intimate contact with CDs (Uekama, Hirayama, & Irie, 1998). However, when compared with other absorption-promoting agents and preservatives used commonly in nasal formulations, CDs exert a rather mild and reversible effect on the surface morphology of nasal mucosa

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and the ciliary beating (Adriaens, Voorspoels, Mertens, & Remon, 1997; Marttin, Verhoef, Romeijn, Zwart, & Merkus, 1996; Romeijn, Verhoef, Marttin, & Merkus, 1996). An additional challenge is the aqueous solubility of β-CD (1.8 g/100 mL at 25 °C), which is often insufficient at therapeutic doses (Gupta, Agashe, Asthana, & Jain, 2006). Therefore, it is essential to further decrease their harmful effects on the mucosal tissue and increase the solubility of β-CD.

Hyperbranched polyglycerols (HPGs) are readily available, well-defined polymer with dendritic branching obtained by controlled anionic polymerization of glycidol (Sunder, Hanselmann, Frey, & Mulhaupt, 1999; Sunder, Mulhaupt, Haag, & Frey, 2000). Moreover, they are promising biomaterials owing to their high water solubility, chemical reactivity, high biocompatibility (as high as that of PEG), and thermally and oxidatively stability (more stable than PEG) (Siegers, Biesalski, & Haag, 2004). Furthermore, the multiple free hydroxyl groups of HPG can be further functionalized with various groups for specific use like many other hyperbranched polymers (Calderón, Warnecke, Gräser, Haag, & Kratz, 2008; Gottschalk, Wolf, & Frey, 2007; Jones, Gao, & Leroux, 2008). It has been reported that hydrophobically derivatized hyperbranched polyglycerols are mucoadhesive, since the surface of this dHPG contains numerous hydroxyl groups and PEG chains (Mugabe et al., 2008). Additionally, in the previous report, we had designed an effective protein delivery system using HPG, which was functionalized with polylactic acid (Gao et al., 2009).

In the present study, we developed a novel nanoparticle (NP) system based on the coupling of β-CD and HPG with the objective of exploring its application as a drug delivery vehicle for insulin. High loading capacity (LC) and encapsulation efficiency (EE) were achieved. The physicochemical properties of the insulin-loaded NPs and the *in vitro* release of insulin were evaluated. Furthermore, the ability to enhance the nasal absorption of insulin was investigated by determining the decrease in blood glucose levels following nasal administration. Finally, the interaction of HPG-*g*-CD NPs with the nasal epithelium was investigated by confocal laser scanning microscopy (CLSM).

2. Materials and methods

2.1. Materials

Glycidol was purchased from Shenyang Jinjiuqi Chemical Co., Ltd. (Shenyang, China). 4-Toluene sulfonyl chloride (TsCl) was recrystallized in petroleum ether before use. Dimethyl sulfoxide (DMSO) and 1,2-ethylenediamine (EDA) were freshly distilled with anhydrous magnesium sulfate and sodium sulfate, respectively. β-CD was obtained from the Sinopharm Chemical Reagent Co., Ltd., China. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was purchased from J&K-Acros Chemical Ltd. (China) without further purification. The dialysis membrane (3.5 kDa and 8 kDa cutoff) was purchased from Shanghai Green Bird Science and Technology Co., Ltd. (Shanghai, China). Pure crystalline porcine insulin (with a nominal activity of 28 IU/mg) was obtained from Xuzhou Wanbang Biochemical Co., Ltd. (Jiangsu, China) and used without further purification. Fluorescein isothiocyanate (FITC) was purchased from Tianjin Lianxing Biotechnology Co., Ltd. (Tianjin, China). The other chemicals were of analytical grade and used as received. The animal experiments had been proved by animal ethical committee in Experimental Animal Center of Tianjin.

2.2. Synthesis of HPG-g-CD

HPG, with a molecular weight of 70 kDa (M_w/M_n = 2.0) was synthesized by the anionic polymerization of glycidol in the presence of alkoxides according to the previous published method (Sunder et al., 1999). The HPG-*g*-CD copolymers were synthesized by the reaction of amine and mono-tosylated β-CD with some modifications (Arima, Kihara, Hirayama, & Uekama, 2001; Kihara, Arima, Tsutsumi, Hirayama, & Uekama, 2003; Kojima, Toi, Harada, & Kono, 2008) and the structure of the copolymer was analyzed by ^{1}H NMR (Varian Unity-plus 400 NMR Spectrometer) and FTIR (FTS-6000, Bio-Rad Co.) (Zhang et al., 2011). By changing the mass ratio of HPG to β-CD, samples with different β-CD grafting ratio were obtained, named HPG-*g*-CD1, HPG-*g*-CD2 and HPG-*g*-CD3, respectively. The apparent amount of β-CD was measured by the previous method (Zhang et al., 2009) and the results are shown in Table 2. However, the molecular weights of the HPG-*g*-CD copolymers failed to be determined by gel permeation chromatography (GPC), owing to the strong interaction of hydroxyl groups in the β-CD ring with GPC column (Zhou et al., 2009).

2.3. Insulin-loaded NPs preparation

Various concentrations of polymer (0.1–4 mg/mL) and insulin (0.5 and 2 mg/mL) aqueous solutions were prepared. 2 mL of insulin solution was slowly added to the same volume of polymer solution under magnetic stirring at room temperature. The solution was incubated overnight at room temperature. The NP suspension was centrifuged at 16,000 × *g* for 30 min, and the resultant NPs were freeze-dried.

2.4. Characterizations of the HPG-g-CD NPs

The hydrodynamic diameter (D_H) and size distribution of NPs were determined by dynamic light scattering (DLS) using a dynamic light scattering particle size analyzer (Brookhaven, INNDVO300/BI900AT) at 25 °C. The zeta potential of the NPs was measured using a zeta potential meter (Zetasizer 3000HS, Brookhaven) at 25 °C. The morphological characteristic of the insulin-loaded NPs was determined by transmission electron microscopy (TEM, Philips, EM400ST).

2.5. Stability of the insulin-loaded HPG-g-CD NPs

In order to evaluate the colloidal stability of the insulin-loaded HPG-*g*-CD NPs in acetate buffer (pH 4.0), the variation of hydrodynamic diameter (D_H) with time at 25 °C was assessed. Size measurements at given time intervals were recorded using a dynamic light scattering particle size analyzer (Brookhaven, INNDVO300/BI900AT).

2.6. Evaluation of the insulin-loading capacity of the NPs

The association capacity of insulin was determined after isolating the NPs from the NP suspension containing free insulin, which was achieved by three cycles of dispersion–recentrifugation (16,000 × *g*, 30 min, 4 °C). The amount of free insulin in the collected supernatant was measured by the Bradford method using a UV spectrometer (Shimadzu UV-2550) at 595 nm (Bradford, 1976). The EE and LC of insulin were calculated using the following equations:

$$\text{EE}\ (\%) = \frac{\text{Total insulin} - \text{free insulin}}{\text{Total insulin}} \times 100\% \quad (1)$$

$$\text{LC}\ (\%) = \frac{\text{Total insulin} - \text{free insulin}}{\text{NPs weight}} \times 100\% \quad (2)$$

2.7. In vitro dug release

Insulin release from the HPG-*g*-CD NPs was analyzed by incubating insulin-loaded NPs (5 mg) at 37 °C in 2 mL of phosphate buffer solution (PBS, 0.1 M, pH 7.4) or acetate buffer (0.1 M, pH 4.0) while