Preparation and characterization of hydroxyapatite nanoparticles carrying insulin and gallic acid for insulin oral delivery

Yuxiao Zhang, BS\textsuperscript{a,1}, Li Zhang, MS\textsuperscript{a,1}, Qing Ban, BS\textsuperscript{a}, Jian Li, BS\textsuperscript{b}, Chu-Hua Li, PhD\textsuperscript{a}, Yan-Qing Guan, PhD\textsuperscript{a,b,*}

\textsuperscript{a}School of Life Science, South China Normal University, Guangzhou 510631, China
\textsuperscript{b}MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, College of Biophotonics, South China Normal University, Guangzhou, China
\textsuperscript{c}Joint Laboratory of Laser Oncology with Cancer Center of Sun Yet-sen University, South China Normal University, Guangzhou, China

Received 7 September 2017; accepted 10 November 2017

Abstract

Although nanoparticles carriers for oral delivery of insulin have been researched for many years, this method still fails to solve issues with toxicity, biocompatibility, and degradability in the organism. We therefore developed an innovative conjugation system to solve this problem. Nano hydroxyapatite (HAP) particles were used as the core, then polyethylene glycol (PEG) was wrapped onto the surface of hydroxyapatite, and, finally, insulin (INS) and gallic acid (GA) were conjugated with PEG. PEG functionalized HAP was increased the hydrophilicity of the nanoparticles, also protected them from degradation in the gastrointestinal (GI) tract. Most importantly, the \textit{in vivo} absorption of nanoparticles in rat small intestines revealed that HAP-PEG-GA-INS was absorbed by the small intestine epithelium. The blood glucose of the type 1 diabetes (T1D) rats that were given intragastrically HAP-PEG-GA-INS showed an obvious downward trend. Overall, we synthesized a safe, non-toxic, and effective oral insulin delivery system.

© 2017 Elsevier Inc. All rights reserved.

Keywords: Diabetes; Oral delivery system; Nanoparticles; Hydroxyapatite

The insulin, which is required for the metabolism of normal sugar, protein, and fat in the human body, is an essential hormone produced by the pancreas. Diabetes occurs when the pancreas does not produce and secrete enough of this hormone.\textsuperscript{1} Type 1 diabetes is characterized by its onset in young age.\textsuperscript{2} At present there is no cure for diabetes. People with type I diabetes mellitus (insulin-dependent) depend on exogenously administered insulin for survival.\textsuperscript{3} However, frequent-injection remains a painful treatment leading to needle phobia and patient noncompliance. Global interest to develop non-invasive, alternative methods to deliver macromolecule drugs, such as insulin, continues to grow.\textsuperscript{4} The oral administration, in recent years, has emerged as potential alternatives to the parenteral way.\textsuperscript{5,6}

Abbreviations: HAP, hydroxyapatite; PEG, polyethylene glycol; INS, insulin; GA, gallic acid; GI, gastrointestinal; T1D, type 1 diabetes; NPs, nanoparticles; DMSO, dimethyl sulfoxide; DCC, dicyclohexylcarbodiimide; EDC, 1-(3-dimethylamino propyl)-3-ethylcarbodiimide; STZ, streptozocin; DMAP, dimethylaminopyridine; NHS, N-hydroxy succinimide; MTT, 3-(4,5)-dimethylthiaiazol-2-yl)-3,5-di-phenyltetrazoliumromide; EDTA, ethylenediaminetetraacetic acid; DMEM, Dulbecco minimum essential medium; DLS, dynamic light scattering; DG, degree of grafting; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; DAPI, 4',6-diamidino-2-phenylindole; H&E, hematoxylin and eosin; PAS, periodic acid Schiff technique; TGA, thermogravimetric analysis; FBS, fetal bovine serum.

Conflict of interest: The authors declare no conflict of interest.

Source of funding: The expenses of this work were supported by the National Natural Science Foundation of China (31370967 and 31170919), the Guangdong Province Universities and Colleges Pearl River Scholar Fund Scheme (2014), China, the Science and Technology Planning Project of Guangdong Province (No.2015A020212031), China.

*Corresponding author at: MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, College of Biophotonics, & Joint Laboratory of Laser Oncology with Cancer Center of Sun Yet-sen University, South China Normal University, Guangzhou 510631, PR China.

E-mail address: guanyq@scnu.edu.cn (Y.-Q. Guan).

The two authors contributed equally to this work and should be considered co-first author.
Although it is the most convenient way to treat patients, the oral administration of drugs has its limitations on the delivery of peptide or protein drugs, such as insulin, due to their sensitivity to hydrolysis and enzymatic degradation, rapid clearance from the site of deposition, and poor absorption through the epithelial membrane of the small intestines. In recent years, nanoparticles (NPs) have been found to be promising platforms to improve the absorption of macromolecular therapeutics (such as insulin). Drugs loaded onto NPs can be sheltered from digestion in the GI tract. The permeability of the intestinal mucosa to the drug was also facilitated by the NPs. In addition, some NPs could achieve the para-cellular transportation of macromolecules by opening the tight junctions between intestinal epithelial cells.

However, oral administration of insulin loaded in NPs is still far from satisfactory. The main reason for this might be involved that the nanoparticles are usually constructed from some toxic organic polymer materials. The long-term administration of these nanoparticles might cause many problems such as slow degradation, low biocompatibility, even a range of functional damage to the human organs. Hydroxyapatite is a bioactive and biocompatible material and it might be an ideal drug carrier because of its porosity. Moreover, it is an efficient adsorption material when combined with nucleic acids and proteins. Oxidative stress in diabetes coexists with a reduction in antioxidant status, which can increase the deleterious effects of free radicals. The supplementation with non-toxic free radical scavengers and antioxidants may facilitate the regeneration of β-cells and protect pancreatic islets against the cytotoxic effects of the streptozotocin (STZ). Within this context, the gallic acid (GA) scavenges and antioxidant effects. It also exhibits the anti-inflammatory and anticancer effects. PEG is widely used in targeted drug delivery systems because it is non-toxic, non-immunogenic, and non-antigenic. The hydroxyl at the ends of this molecule makes it easy to be modified and it is often connected with a variety of proteins and drugs, in order to extend its circulation time in blood and reduce the destructive effects of digestive enzymes.

The HAP-PEG-GA-INS NPs in this study is nontoxic, biodegradable, and have good biocompatibility for oral administration. A series of characterization proved that synthesis of nanoparticles was successful. In this work, we developed the HAP-PEG-GA-INS nanoparticles as a new type of non-toxic and degradable oral delivery system, and it showed that even when coupled with PEG, the insulin can still decrease blood glucose level. The in vivo and in vitro experiments of the HAP-PEG-GA-INS nanoparticles confirmed this effect. Furthermore, after the long-term feeding and histopathological observation of diabetic rats, we found that this transport system had no obvious toxic effects.

Methods

Materials

Details of the Materials were described in supplementary materials.

Preparation and characterization of HAP-PGE-GA-INS

Details of the experiments about preparation and characterization of HAP, HAP-PEG, HAP-PEG-GA and HAP-PEG-GA-INS were described in supplementary materials. The schematic diagram of synthesis was showed in Figure 1.

Stability of nanoparticles

The stabilities of the nanoparticles in the following media were tested: enzyme-containing media including pepsin simulated gastric fluid (SGF, pH 1.2) and pancreatin simulated intestinal fluid (SIF, pH 6.8). Samples were incubated at 37 °C with agitation at 100 rpm on a shaker for 2 h. After that, samples were centrifuged (15,000 g, 4 °C, 30 min) and resuspended in water. The zeta potential of the nanoparticles were measured and compared with the initial values to evaluate the stability of the nanoparticles.

Thermogravimetric analysis (TGA, TG8120, Rigaku Corporation, Shibuya-Ku, Tokyo, Japan) was carried out on the dried samples to evaluate thermal stability of nanoparticles. The measurements were done between 25 °C and 900 °C at a heating rate of 10 °C/min. All experiments were carried out in platinum pans in air atmosphere, and Al2O3 powders (10 mg) were used as a reference.

Drug grafting and the release in vitro

In order to investigate the drug grafting rate of GA and INS in different dosing ratios, HAP-PEG and HAP-PEG-GA nanoparticles were reacted with GA and INS at mass ratios of 1:3, 1:6 and 1:9, respectively. The release of INS and GA in vitro from HAP-PEG-GA-INS in SGF and SIF was measured by UV–vis spectroscopy (UV-2405, Shimadzu Corporation, Osaka, Japan) at 214 nm and 260 nm, which is maximum absorption wavelength of INS and GA. The degree of grafting (DG) of insulin on the surface of HAP-PEG-GA-INS was calculated. The original insulin concentration (W1) and the residual concentration (W2) were measured by spectrophotometric method. The grafted efficiency of insulin on the surface of NPs was calculated with following formula: DG (%) = (W1-W2)/W1 × 100%.

Cell culture

Caco-2 and HepG2 cells were obtained from Medical University of Guangdong, and cultured in DMEM supplemented with 10% FBS at 37 °C under 5% CO2 in a humidified atmosphere. When the cells reached 80% confluence, they were removed by 0.25% (w/v) trypsin containing 0.02% (w/v) EDTA in PBS and were cultured on a new tissue culture plate for subculture.

Cytotoxicity assay

Cell viability was measured with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Caco-2 cells were cultured in DMEM at 37 °C and 5% CO2. The cells were subcultured into 96-well plates. A monolayer of cells formed within 36 h, whereupon cells were treated with 0, 12.5, 25, 50, or 100 mg/ml of HAP-PEG-GA-INS for 24 h. After the 24 h treatment, we refreshed the serum-free medium containing MTT 0.5 mg/ml and cells were
دریافت فوری متن کامل مقاله

امکان دانلود نسخه تمام متن مقالات انگلیسی
امکان دانلود نسخه ترجمه شده مقالات
پذیرش سفارش ترجمه تخصصی
امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
امکان دانلود رایگان ۲ صفحه اول هر مقاله
امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
دانلود فوری مقاله پس از پرداخت آنلاین
پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات