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Absorption and distribution of water-soluble hydroxypropyl chitosan in mice after oral administration

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ABSTRACT

Two kinds of water-soluble hydroxypropyl chitosan (HPCS) samples with different weight-average molecular weights (M_w) (HHPCS for higher M_w, 1.43×10^5 Da; LHPCS for lower M_w, 5.71×10^3 Da) were used to investigate their absorption and distribution in mice by oral administration after being labeled by fluorescein isothiocyanate. The result of *in vitro* degradation showed that HPCS degraded more easily in the presence of lysozyme than in pepsin. After oral administration of HPCS, HPCS would undergo a series of degradation and absorption under the action of tissue cells and enzymes in the body. The absorption and distribution of HPCS in organ tissues was significantly influenced by its M_w. With the decrease of M_w, the absorption rate and amount of HPCS increased. In fact, only a small amount of HPCS and its catabolite with low M_w was absorbed by the tissues, and most HPCS was metabolized and excreted out of body rapidly. Additionally, there was some accumulation of HPCS in liver, and so HPCS content in liver and kidney was higher. The whole HPCS plasma concentration was lower instead, due to rapid plasma clearance. The absorbed HPCS molecules still maintained a relatively higher concentration even after 16 h in the tested tissues. The research results gave some valuable data for the application of water-soluble chitosan in food and biomedicine, especially as a potential candidate for drug delivery systems.

1. Introduction

As a natural linear cationic polysaccharide, chitosan (CS) has attracted much scientific and industrial interest in the fields of biotechnology, pharmaceutics, food science, waste water treatment, agricultures and textiles (Elchinger et al., 2015; Mati-Baouche et al., 2014; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Sandford & Stinnes, 1991; Shahidi, Arachchi, & Jeon, 1999), due to its unique bioactivity, including its excellent biodegradability, biocompatibility and nontoxicity (McAlindon, LaValley, Gulin, & Felson, 2000; Muzzarelli, 1993; Peng, Han, Liu, & Xu, 2005). In 1983, CS has been approved as feed additive by Food and Drug Administration (FDA). Especially for the pharmaceutical and biomedicine area, most studies indicated that CS has been considered as one of the most excellent candidates for drug delivery systems to enhance drug absorption, control drug release and target therapy as drug carriers (Agnihotri, Mallikarjuna, & Aminabhavi, 2004; Bansal, Sharma, Sharma, Pal, & Malviya, 2011; Bernkop-Schnürch & Dünnhaupt, 2012; Ravi Kumar et al., 2004; Sonia & Sharma, 2011; Wang, Tao, Zhang, Wei, & Ren, 2010).

Although many studies have been focused on the applications of CS in food engineering and biomedicine, its bioactive properties *in vivo* remained unclear, including bio-distribution, body absorption, biodegradation and toxicity, which were in close relation with drug action, medicine persistence and safety. Chae et al. investigated molecular-weight-dependent body absorption of CS *in vitro* and *in vivo*. CS oligosaccharides showed higher permeation and absorption profiles with negligible cytotoxic effect, which may be considered as a safe and potential candidate for pharmaceutical and biomedical applications (Chae, Jang, & Nah, 2005). Onishi et al. discovered that chitin with 50% deacetylate was highly biodegradable and was excreted quickly, and there was no accumulation in the mice after intraperitoneal administration (Onishi & Machida, 1999). As for CS micro-particles, Shimoda et al. analyzed its bioadhesive and absorptive characteristics (Shimoda, Onishi, & Machida, 2001; Takishima, Onishi, & Machida, 2002). Our previous study also found that the body absorption of CS was in close relation with its molecular weight and water-solubility (Zeng, Qin, Wang, Chi, & Li, 2008). CS with lower molecular weight had better

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solubility and was absorbed more easily by body tissues. For the absorption and distribution of chitosan, it was difficult for chitosan to be located *in vivo*, due to its colorless and no UV absorption, and so it was necessary for chitosan to be labeled using fluorescein. At present, isothiocyanate was used as fluorescein most commonly in most past studies (Chae et al., 2005; Onishi & Machida, 1999; Shimoda et al., 2001; Zeng et al., 2008).

In spite of these superior properties, plain CS has a major drawback: its solubility is poor above pH 6.0 (Qin et al., 2006). Now that most researches indicated that the bioactivity of CS was in close relation with its molecular weight and solubility, it is necessary to investigate the bioactivity of the water-soluble CS, such as carboxymethyl CS, and CS quaternary ammonium salt. Hydroxypropyl CS (HPCS) is another CS derivative with excellent water solubility, which was potentially suitable for drug carrier and food additive (Bansal et al., 2011; Sonia & Sharma, 2011; Wang et al., 2010). In the past few years, the structure, properties and use of HPCS were studied (Dong, Wu, Wang, & Wang, 2001; Peng et al., 2005), but little research about its metabolism *in vivo* was done. The safety evaluation of modified natural products is very important for their applications in food and drug carrier. In this paper, HPCS with different molecular weights was used to investigate its absorption and distribution in mice after oral administration by using fluorescein-labeling method.

2. Materials and methods

2.1. Materials

Initial CS (M_w, 1.86×10^5 Da; degree of *N*-deacetylation, DD, 90%) was supplied by Golden-shell Biochemical Co. LTD, China. Pepsin and lysozyme were purchased from Aladdin Co., whose activities were more than 2,500 units/mg dry weight and 5,000 units/mg dry weight, respectively. Fluorescein isothiocyanate (FITC) was purchased from Sigma Chemical Co. (USA). HPCS (degree of substitution, DS, 1.41) was obtained by etherification of CS with propylene oxide under alkaline conditions according to the previous study (Wang et al., 2014). Phosphate buffer solutions (PBS) were prepared using 0.2 mol/L sodium dihydrogen phosphate and 0.2 mol/L disodium hydrogen phosphate to form pH 5.5 and pH 6.86 solutions. All other reagents were of analytical grade and used directly without any further purification. Kunming female strain mice (4 weeks old) with a body weight ranging from 18 to 22 g were obtained from Hubei Experimental Animal Center (Wuhan, China).

2.2. In vitro degradation of HPCS

In order to understand the metabolism of HPCS in body, its enzymatic degradation was investigated imitating body metabolism using pepsin and lysozyme *in vitro*. Initially, HPCS (1.0 g) was dissolved in 500 mL phosphate buffer solution (pH 5.5), and then pepsin or lysozyme (10 mg) was added into the solution. The solution with and without enzymes was incubated at 37 °C, and the viscosity change was checked using the Ubbelohde viscosimeter at predetermined time.

2.3. Preparation of HHPCS and LHPCS samples

HPCS (10 g) was completely dissolved in 500 mL distilled water. UF-membrane of 10 kDa was used to separate the solution. The residue solution, which did not pass through the membrane, was precipitated by adding excessive acetone with 6 times volume. The solid was filtrated and washed with acetone 3 times to obtain HPCSsample with higher M_w (HHPCS).

Lysozyme (0.1 g) was added into 500 mL 2 wt% HPCS aqueous solution. The solution was initiated under stirring at 37 °C for 8 h, and then the mixture was boiled for 10 min to deactivate the enzyme, and the enzyme residue was removed by filtration. The filtrate was separated by using UF-membrane. The fraction which passed through 10 kDa membrane but not 1 kDa membrane was precipitated in excessive acetone with 6 times volume, and the product was washed with acetone 3 times to get HPCS samples with lower M_w (LHPCS). At last, the obtained HHPCS and LHPCS samples were dried by lyophilization.

The average molecular weight of the obtained HPCS samples was determined by gel permeation chromatography according to the previous paper (Qin, Du, Xiao, Li, & Gao, 2002). The M_w of HHPCS and LHPCS were 1.43×10^5 Da and 5.71×10^3 Da, respectively. And their polydispersities were 1.87 and 1.16, respectively.

2.4. FITC labeling of HPCS (FITC-HPCS)

HPCS was labeled with FITC according to the previous study (Zeng et al., 2008). HPCS (0.6 g) was completely dissolved with double distilled water to form 2 wt% aqueous solution, and then methanol with 1.5 times volume was added under continuous stirring to form co-solvent system. Afterwards, a predetermined amount of FITC (HPCS/FITC, 60/1, w/w) dissolved in a small amount of acetone (about 2 mL) was added to the HPCS aqueous solution. The mixture was vigorously stirred for 24 h in dark at room temperature, and FITC-HPCS was obtained by precipitating the mixture in excess acetone with 6 times volume. The obtained FITC-HPCS was washed with acetone 6 times until there was no more visible absorption at 490 nm in filtrate with reference to FITC. Finally, the FITC-HPCS was obtained by lyophilization.

The absorbance strength of 5 mg/mL FITC-HPCS water/methanol (1/1.5, V/V) solution at the band of 490 nm was obtained using UV spectrophotometer. The content of FITC in FITC-HPCS (also known as labeling yield) was determined according to FITC standard curve using absorbance strength versus FITC concentration (Chae et al., 2005; Zeng et al., 2008). FITC contents of labeled HHPCS (FITC-HHPCS) and LHPCS (FITC-LHPCS) were 1.16% and 2.43%, respectively.

2.5. In vivo HPCS distribution test

Female mice (18–22 g body weight) were divided into groups with five mice each randomly. Before the administration of HPCS, the mice were fasted for 12 h but given water ad libitum, and then were administered 20 mg/mL FITC-HPCS aqueous solution through an oral gavage tube. The total volume of the administrated HPCS was 0.20 mL (at the dose of 200 mg/kg). The blood, liver, kidney, thymus, lung, heart and spleen were collected after the mice were sacrificed at the predetermined time. The blood samples from eyeballs were used directly after been suspended at room temperature overnight. The other various tissues were homogenized using glass homogenizer together with physiological saline (0.9 wt% NaCl solution), and then were centrifuged at 4,200 rpm for 20 min to separate the insoluble solids. Next, the 0.50 mL obtained sample solutions were added to 3.50 mL phosphorous buffer solution (pH 6.86). The fluorescence intensity of the resulting solutions was measured with an excitation (EX) wavelength at 490 nm using FLS 920 Fluorescence Spectrometer. The obtained fluorescence intensity at 520 nm of the emission (EM) wavelength was normalized with the standard FITC-HPCS solution, and the FITC-HPCS content of each tissue was determined from the corrected concentration and the tissue weight. The plotted data were the mean $\pm$ SD ($n=3$).