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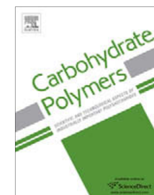

Preparation of aspirin and probucol in combination loaded chitosan nanoparticles and in vitro release study

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ABSTRACT

We design and develop chitosan nanoparticles which load two different drugs simultaneously. Aspirin (acetylsalicylic acid, ASA), a hydrophilic drug and probucol (PRO), a hydrophobic drug, are chosen as typical drugs, which are widely used to treat restenosis. The drug loaded chitosan nanoparticles are prepared by gelation of chitosan with tripolyphosphate (TPP) by ionic cross-linking. The physicochemical properties of nanoparticles are investigated by FTIR, transmission electron microscope (TEM), scanning electron microscopy (SEM) and differential scanning calorimetry (DSC). The images show that these particles are spherical in shape with ASA being in the amorphous phase, while PRO is crystalline. The properties of chitosan nanoparticles such as encapsulation capacity and controlled release behaviors of ASA and PRO are evaluated. Experimental results indicate that the loading capacity (LC), encapsulation efficiency (EE) and ASA and PRO release behaviors are affected by several factors including pH, concentration of TPP, chitosan molecular weight (MW) and ASA initial concentration as well as PRO. In vitro release shows that the nanoparticles provide a continuous release. Entrapped ASA is released for more than 24 h and PRO lasts longer for 120 h.

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1. Introduction

Percutaneous transluminal coronary angioplasty (PTCA) is a promising method for curing obstructed coronary artery disease. PTCA procedures include balloon dilation, excisional atherectomy, endoluminal stenting and laser ablation (Baim, 1992). However, revascularization induces thrombosis and neointimal hyperplasia, which in turn cause restenosis in 30–40% of coronary arteries within 6 months after successful balloon angioplasty (Nobuyoshi, Kimura, & Ohishi, 1991). Patients undergoing PTCA require additional surgical intervention because of a combination of factors including elastic recoil, thrombosis, vessel remodeling, local tissue inflammation and neointima formation (Currier & Faxon, 1995). In general, the pathogenesis of restenosis is multifactorial. What is more, the patients, suffering restenosis, show several symptoms than could be controlled by employing multidrugs. So combination dosing is usually adopted in clinical therapy. There are a variety of drugs (e.g. aspirin, probucol, rapamycin) which are used for clinical treatment. Aspirin (ASA) as a hydrophilic drug is often used due for its anti-atherosclerotic effect (Kato et al., 2005). Probucol (PRO), a hydrophobic drug may prevent restenosis by exerting an anti-oxidative effect (Watanabe, Sekiya, Ikeda, Miyagawa, & Hashida, 1996). As common drugs used in anti-restenosis, ASA and PRO chosen in this study.

Furthermore, an effective anti-restenotic therapy should combine sufficiently high and sustained drug levels at the injury site with minimal systemic and local toxicity (Banai et al., 2005). Drug loaded nanoparticles offer the advantage of high tissue uptake and protracted drug residence at the injury site (Banai et al., 1998; Fishbein et al., 2000, 2001; Guzman et al., 1996). There has been considerable interest in developing chitosan nanoparticles as effective drug delivery devices (Janes, Calvo, & Alonso, 2001). Chitosan is a linear polysaccharide containing two β-1,4-linked sugar residues, *N*-acetyl-D-glucosamine and D-glucosamine, distributed randomly along the polymer chain. It is obtained commercially by partial de-*N*-acetylation of chitin (Thierry, Winnik, Merhi, Silver, & Tabrizian, 2003). It is a biodegradable, biocompatible, naturally occurring polymer. Chitosan is one of the major components used in vascular surgery, tissue culture and tissue regeneration as a hemostatic agent (Kolhe & Kannan, 2003; Li, Yun, Gong, Zhao, & Zhang, 2006). Chitosan scaffolds have been investigated for use in tissue engineering (Madihally & Matthew, 1999). Even though the discovery of chitosan dates from the 19th century, it has only been over the last two decades that this polymer has received attention as a material for biomedical and drug delivery applications because of its desirable biological properties.

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Chitosan nanoparticles showed high cytotoxic activity toward tumor cells, while low toxicity against normal human liver cells (L-02) (Qi, Xu, Jiang, Li, & Wang, 2005). Chitosan nanoparticles show high sorption capacity and anti-bacterial activity (Qi & Xu, 2004; Qi, Xu, Jiang, Hu, & Zou, 2004). The unique cationic character of chitosan nanoparticles could provide higher affinity with negatively charged biological membranes and site-specific targeting in vivo (Qi et al., 2004). Particle size also substantially increases their anti-tumor efficacy when chitosan nanoparticles are applied by intravenous injection (Qi et al., 2005). The unique character with positive charge and small particle size of chitosan nanoparticles is responsible for their in vivo efficacy (Qi & Xu, 2006). Moreover, it is reported that chitosan can suppress the proliferation of vascular smooth muscle cells, consequently, preventing restenosis of rabbit (Joseph, 2006; Lim et al., 2005). Therefore, drug loaded chitosan nanoparticles might be valuable in the treatment of restenosis.

In this paper, we report the study of the drug release behavior of ASA/PRO from nanoparticles, ASA and PRO combined loaded chitosan nanoparticles have been prepared based on an ionic gelation process. We have characterized and compared the properties of chitosan nanoparticles loaded with combined drugs under different preparation conditions, pH value and concentration of TPP. The drugs in vitro release, LC and EE of drugs are also explored with a view to understanding the effects of pH and concentration of TPP on molecular interactions interactions between the two drugs. Thus, we can modulate their encapsulation capability and release rate by adjusting the molecular and formation parameters.

2. Materials and methods

2.1. Materials

Chitosan with different MW (21, 40, 67 kDa and degree of deacetylation was 90%) are obtained from Dacheng Biotech. Co. Ltd. (Weifang, People's Republic of China). Aspirin is obtained from Lunan Pharmaceutical Co. Ltd. (Linyi, China). Probucol is from Qilu Pharmaceutical Co. Ltd. (Jinan, China). Sodium tripolyphosphate (TPP) and other reagents are all of analytical grade.

2.2. Preparation of ASA and PRO combined drugs loaded chitosan nanoparticles

Chitosan solutions (2.5 mg/mL, 25 mL) are prepared by dissolving chitosan in 1% acetic acid. ASA is added into the solution with different concentration (0.8, 1.0 and 1.2 mg/mL). After dissolving completely, Tween-80 (2% v/v) is added as a surfactant. PRO (7.5, 10.0 and 12.5 mg) is dissolved in CH_2Cl_2 and then this oil phase is mixed with aqueous phase (chitosan solution contained aspirin) by stirring vigorously for 20 min. CH_2Cl_2 is chosen because of its ability to diffuse into the aqueous phase at a rapid rate facilitating particles formation upon evaporation. The ratio of oil and aqueous phase is 1:10. TPP solution (10 mL) is dropped into O/W emulsion under magnetic stirring. After 1 h of cross-linking, nanoparticles are isolated by centrifugation at 9000 rpm for 30 min.

2.3. Morphological characterization

The surface morphology of nanoparticles is observed by TEM and SEM. For TEM, the nanoparticles solution is dropped on copper grids and dried overnight at room temperature for viewing (JEM-100CX, JEOL, Japan). Samples of frozen dried nanoparticles are mounted on metal stubs, gold coated under vacuum and then examined on a S-2150 SEM (Hitachi, Japan).

2.4. FTIR analysis

The nanoparticles solution is centrifuged at 9000 rpm for 30 min. Supernatants are discarded and drug loaded chitosan nanoparticles are freeze-dried for 24 h at −34 °C, followed by a gradual increase in temperature until 20 °C, using a BETA1-8 freeze-dryer (BETA, N/A) ($n = 3$). The IR spectrum of samples is recorded on a Fourier Transform Infrared Spectrometer 430 (Perkin-Elmer, USA).

2.5. DSC analysis

A differential scanning calorimeter (DSC), Model Perkin-Elmer PYRIS I, was used. Each freeze-dried sample (5–10 mg) is run at a scanning rate of 10 °C/min under nitrogen atmosphere. The temperature for the scan ranged from 20 to 180 °C.

2.6. Evaluation of drug loading efficiency

The encapsulation efficiency and loading capacity of nanoparticles are determined by the separation of nanoparticles from the aqueous medium containing free drug by centrifugation at 9000 rpm for 30 min. The amount of free ASA and PRO in the supernatant is measured with a spectrophotometer at 242 and 298 nm, separately. Dilutions of samples and calibration curves are performed in phosphate buffered saline (PBS pH 7.4). The encapsulation efficiency ($EE_{1,2}$) and loading capacity ($LC_{1,2}$) of ASA and PRO of the nanoparticles are calculated as follows:

$$EE_{1,2} = \frac{T_{1,2} - F_{1,2}}{T_{1,2}} \times 100, \quad LC_{1,2} = \frac{T_{1,2} - F_{1,2}}{W_N} \times 100$$

$T_{1,2}$ are total amount of ASA and PRO. $F_{1,2}$ are amount of free ASA and PRO. W_N is the nanoparticles weight. All measurements are performed in triplicate.

2.7. Evaluation of in vitro drug release

The ASA and PRO combined drugs loaded nanoparticles separated from 18 mL suspension are placed into test tubes with 6 mL of 0.2 mol/l phosphate saline cushion liquid (PBS), and incubated at 37 °C under stirring. At varying time points, supernatants are isolated by centrifugation. Samples (3 mL) are removed and replaced by fresh medium with the same volume. The amount of released ASA and PRO are analyzed with spectrophotometer (PE, America) at 298 and 242 nm, respectively.

2.8. Statistical analysis

All experiments are repeated a minimum of three times and measured in triplicate. Results reported are means ± SD, unless otherwise noted. Statistical significance is analyzed using Student's *t*-test. Differences between experimental groups are considered significant when *P*-value is less than 0.05.

3. Results and discussion

3.1. Formation and characterization of combined drugs loaded chitosan nanoparticles

The preparation of chitosan nanoparticles, based on an ionic gelation process, involves the mixture of two different phases at room temperature. One phase is an aqueous phase of acetic acid solution containing chitosan and ASA. The other phase is an oil

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