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Injectable chitosan thermogels for sustained and localized delivery of pingyangmycin in vascular malformations

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ABSTRACT

Pingyangmycin (PYM) is an effective drug to treat vascular malformations (VM), but can easily diffuse from the injection site, which will reduce its therapeutic effect and increase side effect. Our study was to evaluate PYM-loaded chitosan thermogels for sustained and localized embolization therapy. It was shown that in vitro release of PYM thermogels could be delayed up to 12 days. The results measured by MTT assay showed that PYM thermogels could inhibit proliferation and induce apoptosis of EA. hy926 cells in a concentration and time dependent manner. In vivo pharmacokinetics study demonstrated that compared with PYM injections, PYM thermogels had a better sustained delivery of PYM. Macroscopic observation and histological examination of rabbit ear veins displayed that after administration with PYM thermogels for 18 days, obvious venous embolization and inflammatory response could be found. These results indicate that PYM thermogels is likely to achieve excellent prospects for VM treatment.

1. Introduction

Vascular malformations (VM), which often occurred in the oromaxillofacial regions, are common diseases and their treatment remains difficult. Different methods including sclerotherapy, embolization, cryosurgery, radiotherapy, laser cosmetic surgery have been implemented in the treatment of VM (Hontanilla et al., 2013; Merrill et al., 2011; Scharpfenecker et al., 2009; Zhang et al., 2013; Zhao et al., 2013). Optimal therapeutic strategies are well connected with the size and position of the lesion, for example, respiratory tract lesion are normally treated by laser coagulation and surgical excision method is the first choice if the lesion is surgically resectable (Chien et al., 2013; Lim et al., 2013; Ring et al., 2013). For many years, the therapy of VM mostly relied on conventional plastic surgery which has lots of disadvantages such as poor compliance, obvious scar and high risk due to the intricate blood vessel distribution in the oral and maxillofacial areas (Wu et al., 2006). Moreover, the possibility of facial distortion after surgery seriously affects the psychological health of patients (Zhao et al., 2012). As an alternative to traditional surgery method, interventional embolization therapy has been applied more precisely and conveniently to obstruct blood vessels for the treatment of VM in the past few years (Clarencon et al., 2012; Katz et al., 2012; Nugent et al., 2013; Patsalides et al., 2011). Recently, chemoembolization, which connects the benefits of therapeutic drugs and embolic materials, has become a promising therapy for VM (Almefty et al., 2013; Liu et al., 2006).

Pingyangmycin (PYM), as also called Bleomycin A5, is a water soluble glycopeptide antitumor antibiotic produced by streptomycete which was isolated from the soil of Pingyang, a city of China. As an effective sclerosant, PYM has been widely used in the Far East for treatment of VM with an exact therapeutic effect (Chen et al., 2010; Jia et al., 2014). Intravenous and local injection of PYM could result in injury and disengagement of endothelial cells, thicken the vascular wall, and occlude the blood vessel (Luo and Gan, 2013). Nowadays, freeze dried PYM powders for injection are the only marketed formulation. However, short half life and many side effects, especially pulmonary toxicity have restricted and hindered

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its wider application (Pan et al., 2009). So, the study of a sustained and localized release formulation of PYM in VM is very meaningful for improving the therapeutic effect and minimizing the risk of side effects. There are a few reports about the combined use of PYM and biodegradable embolic agents, such as Bovine serum albumin microspheres, Zein/Zein–sucrose acetate isobutyrate in situ gels and PLGA microspheres (Gao et al., 2007; Han et al., 2010; Wang et al., 2007, 2008).

Hydrogels are polymeric networks with large amounts of water, which have drawn much attention due to their biocompatibility and stability (Peng et al., 2013). Recently, in situ formation by simple sol–gel transition without any chemical reaction is one of reseach highlights in hydrogels, which makes it more practicable to apply hydrogels for biomedical engineering and drug delivery (Jeong et al., 2002; Yeon et al., 2013). The injectable biodegradable thermogel system has many advantages including in situ depot formation without surgical procedures, convenience of drug loading and dosage modifications (Bhattarai et al., 2005; Park et al., 2012; Yun et al., 2012). Chitosan (CS) was widely used in pharmaceutics and tissue engineering due to its low toxicity, good biodegradability and biocompatibility. An interesting thermogelation of a mixture of CS and glycerol phosphate disodium (GP) was reported previously (Chenite et al., 2000; Supper et al., 2014). The CS/GP thermogels were applied as injectable scaffold materials in cartilage regeneration, angiogenesis and bone repair (Cheng et al., 2011). Local and sustained delivery of paclitaxel from the CS/GP thermogels was investigated by intratumor injection into EMT6 tumor-bearing mice, and the results showed that the paclitaxel thermogels were more efficient in inhibiting the growth of tumors and less toxic than Taxol injections (Ruel-Gariepy et al., 2004).

In the present study, in order to develop a novel embolic agent for chemoembolization therapy of VM, we selected CS/GP mixed system as a vehicle for PYM thermogels that were injected as a solution and then changed into a semisolid embolic agent which could occlude blood vessels to interrupt the nutrition supply to VM and slowly release PYM to keep an effective therapeutic concentration. Toward this end, PYM-loaded CS/GP thermogels were prepared and investigated in vitro and in vivo.

2. Materials and methods

2.1. Materials

PYM was purchased from Liaoyuan Dikang pharmaceutical Co., Ltd. (Jilin, China) and CS with a deacetylation degree of 95% and molecular weight of 50 kDa was supplied by Jinan Haidebei Marine Bioengineering Co., Ltd. (Shandong, China); GP was purchased from Sinopharm Chemical Reagent Co., Ltd (Shenyang, China). All other chemicals and solvents used were of analytical grade. Rabbits were provided by Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The human vascular endothelial cell line EA.hy926 was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). DMEM Medium was obtained from GIBCO Co. (NY, USA) and fetal bovine serum (FBS) was purchased from Beyotime Biotechnology Co. (Shenyang, China).

2.2. Preparation of PYM thermogels

Typically, the chitosan (200 mg) powder was slowly added to 0.1 mol/L acetic acid (8 mL) under stirring, and then the mixture was continuously stirred overnight to make a clear solution. Then the chitosan solution was chilled to 4 °C in an ice bath. GP (1 g) and PYM (20 mg) were dissolved in 1 mL purified water separately, and chilled along with the chitosan solution to 4 °C for 10 min. The GP solution was added dropwise into the chitosan solution with agitating and then they were mixed for 10 min. Finally, the formulation was obtained by adding PYM solution to the CS/GP solution under stirring for another 10 min.

2.3. In vitro evaluation of gelation time and viscosity of PYM thermogels

The time required to transform a solution into a gel at 37 °C (defined as gelation time) was examined using a simple vial tilting method. When the vial of PYM thermogels was inverted, the solution phase was regulated as flowing liquid and the gel state as non-flowing gel. PYM CS/GP thermogels (3 mL) prepared following the steps above were added into 10 mL vial to evaluate the sol–gel transition behavior in a water bath of 37 °C. At the predetermined time interval, the vial was taken out and inverted to observe the state of the thermogels. No flow within 0.5 min of inverting the vial of PYM thermogels was the standard for the complete gel state (Chung et al., 2002). The viscosity of PYM thermogels was determined at 37 °C using a Digital Viscometer (NDJ-8S, Shanghai Precision Instrument Co., Ltd., China), and the effect of the concentration of GP, CS and PYM on the gelation time and viscosity (before and after sol–gel transition) of PYM thermogels was investigated, respectively.

2.4. Rheological measurements

The rheology study of the PYM thermogels was carried out at different temperatures using a AR2000ex rheometer (TA Co., Ltd., USA) equipped with a parallel plate geometry (plate diameter = 40 mm, gap = 0.45 μm and stress = 10 Pa) for the oscillatory shear rheological measurements and 5 mL formulation sample prepared following the steps above was needed (Martinez-Ruvalcaba et al., 2007). In variable temperature mode, the changes in elastic modulus (G′) and viscous modulus (G″) were recorded as a function of temperature. The oscillatory frequency was fixed at 1.0 Hz during the measurements. The sample of PYM thermogels was placed on the plate of the rheometer and the temperature changed from 15 °C to 45 °C with a rate of 2 °C/min (Nisbet et al., 2006).

2.5. In vitro release studies

First, 3 mL of the PYM thermogels were injected into 20 mL glass vials (diameter 20 mm) and then kept warm in an 37 °C water bath for half an hour so that they could be transformed into gel completely. Then 15 mL phosphate buffer solution (pH 7.4) (PBS) containing NaN_3 (0.05%, w/v) and lysozyme (1.0%, w/v) were added into the vials. The dissolution system was shaken in an incubator at 50 rpm and 37 °C. The release medium was all collected at predetermined time intervals for analysis and replaced with same amount of fresh dissolution medium. The concentration of PYM in the release medium was assayed by a high performance liquid chromatography (HPLC) according to the previously reported method (Gao et al., 2007). All the in vitro dissolution tests were carried out in triplicate, and the accumulation release amount of PYM from thermogels was calculated.

2.6. In vitro cytological studies

2.6.1. Cell culture

EA.hy926 cells were grown in DMEM medium, complemented with 10% FBS and $NaHCO_3$ (1.5 g/L), at 37 °C in a humidified 5% CO_2 incubator. Treatments were performed on 80–90% confluent cells.

2.6.2. In vitro cell viability

MTT assay was utilized to evaluate the in vitro cell viability of EA.hy926 incubated in the presence of CS thermogels, PYM