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Phytantriol-based inverted type bicontinuous cubic phase for vascular embolization and drug sustained release

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ABSTRACT

The potential feasibility of using phytantriol-based inverted type bicontinuous cubic phase as an embolization agent and sustained release system was evaluated in this study. In the ternary phytantriol–water–ethanol system, when water content was less than 30% (w/w), the injectable isotropic solution was formed and could transform into the bicontinuous cubic phase upon contacting the dissolution/body fluids. The transformation of the isotropic solution was confirmed by polarized light microscopy (PLM), small angle X-ray scattering (SAXS), resonance Raman spectroscopy, and rheological measurements. The *in vitro* dissolution results showed that the release was sustained for up to 30 days and was affected by drug loading and the initial compositions of isotropic solutions. *In vivo*, the embolization study was performed with normal rabbits using transcatheter arterial embolization technique and was monitored under digital subtraction angiography (DSA). The angiographical results showed that the hepatic artery was successfully embolized with phytantriol cubic phase. Therefore, with the vascular embolization and sustained release characteristics, the phytantriol-based inverted type bicontinuous cubic phase could be used for arterial transcatheter chemoembolization on hepatocellular carcinoma.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common liver cancer (Guan et al., 2004), with about 500,000–1,000,000 new patients being diagnosed annually all over the world. Several non-surgical modalities have been developed for the treatment of HCC (Lau et al., 2009), including transarterial and chemoembolization (TACE) as an attractive alternative to surgical treatment (Morimoto et al., 2008; Yoon et al., 2003). TACE involves intra-arterial administration of chemotherapy combined with arterial embolization. It could reduce arterial inflow, diminish washout of the chemotherapeutic agent, and decrease systemic exposure (Pleguezuelo et al., 2008).

Various materials have been used for decades in TACE (Li et al., 2005; Oowaki et al., 2000), of which the liquid adhesive polymer-based solution has received a considerable attention worldwide (Seron et al., 2009; Oowaki et al., 2000). However, the solutions usually contain dimethyl sulfoxide (DMSO), which is a powerful solvent and hence have major drawbacks related to this solvent (Seron et al., 2009). To overcome this limitation, it was supposed to develop a polar amphiphilic material that self-assembles in water and could act as a novel cohesive embolic agent without using DMSO.

Cubic liquid crystalline phase, which is formed by polar amphiphilic material, has caused much attention in the fields of drug delivery system (Shah et al., 2001; Chang and Bodmeier, 1997a,b; Yaghmur et al., 2005). The unique structure of cubic liquid crystalline phase has been extensively studied and explored in a wide range of applications (Geraghty et al., 1996; Lee et al., 2009; Rizwan et al., 2009; Lara et al., 2005; Yaghmur and Glatter, 2009). In this study, it was proposed that its resemblance of biomembranes, stiffness and extremely high viscosity might make cubic liquid crystalline phase an excellent candidate for specific use as a plug that obstructs the lumen in TACE. However, due to the high viscosity, the cubic liquid crystalline phase is not practicable for direct administration. As a result, a low viscosity precursor system should be prepared that forms cubic liquid crystalline phase on exposure to excess solution/body fluids (Fong et al., 2009). According to previous publications (Chang and Bodmeier, 1998; Wadsten-Hindrichsen et al., 2007), organic solvents, such as ethanol, polyethylene glycol, propylene glycol and *N*-methyl-2-

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pyrrolidone, could significant impact on the structure leading to the formation of low viscous solutions. It has been specially pointed out that water–miscible ethanol has a good compatibility with catheter and is well known as a sclerosing agent in TACE (Seron et al., 2009; Mottu et al., 2000); and the viscosity of ethanol is the lowest one for clinical use among the solvents described above. Besides, as a consequence of the choice of ethanol as the solvent, docetaxel, which is a highly lipophilic drug and produces profound effects on HCC *in vitro* (Xu et al., 2009), could dissolve in the polar amphiphilic material well. Therefore, ethanol was selected as the solvent in this study based on its water-miscibility and still being used in docetaxel commercial formulation and clinical TACE.

Phytantriol, a neutral polar lipid, is well-known as an ingredient in cosmetic products for hair and skin care (Wagner, 1994). However, the recent interest in phytantriol focuses on its lyotropic phase behavior (Lee et al., 2009), which can swell and form cubic liquid crystalline phase when contacting with water (Barauskas and Landh, 2003). Consequently, in this study, an injectable precursor of phytantriol cubic liquid crystalline phase, which induced by ethanol, was investigated as a novel embolic solution and drug carrier.

2. Materials and methods

2.1. Materials

A commercially available grade of phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) was purchased from Wako Japan, with a nominal purity of >97%. Docetaxel was purchased from Yi Kang Si Da Medical Technology Ltd., China. Rabbits were purchased from Central Animal Laboratory of Sun Yat-sen University (Guangzhou, China). All other chemicals used were reagent grade.

2.2. Phase determination

Ternary phase diagram was prepared by firstly mixing ethanol with phytantriol in ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 (w/w). A specific amount of each mixture was transferred into a vial and mixed with water in ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 (w/w) to a total weight of 0.2 g. The preparations were then vortex-mixed homogeneously and centrifuged at 3000 rpm for 20 min, followed by equilibrating for one week at $25 \pm 0.5\,^{\circ}C$. After equilibration, samples were viewed through crossed polarizing filters to examine the mesophases using PLM at $25 \pm 0.5\,^{\circ}C$, and classified according to the visualized textures of the lyotropic mesophases (Rosevear, 1954, 1968). Further confirmation of liquid crystalline phase structure was undertaken using SAXS, which was performed using a Kratky Compact Camera (HMBG, Austria) with Ni filtered Cu Kα radiation (wavelength $\lambda = 1.5418$ Å) generated by an X-ray generator (50 kV × 40 mA). Scattering intensities were plotted versus q-value, which enabled the identification of peak positions. The recorded diffraction patterns were evaluated using 3D-View software (Mbraun. Graz. Austria) without any additional data manipulation.

2.3. Water uptake/swelling study

For swelling study, the binary phytantriol/water systems containing 0% and 10% (w/w) of water were weighed into glass bottles, sealed and stored at $25 \pm 0.5\,^{\circ}C$ for at least one week to equilibrate prior to swelling study.

The uptake of water by the matrices was determined gravimetrically. Amount of 5 ml plasma was gently placed over the equilibrated matrices, which were then shaken horizontally at 100 rpm in $37 \pm 0.5\,^{\circ}C$ water bath. At the predefined time intervals, the plasma was removed and the matrices were blotted with fine weaving paper to remove any remaining liquid and then re-weighed. After re-weighed, fresh 5 ml plasma was again placed onto the surface of the matrices and the matrices were then placed back in the water bath (Rizwan et al., 2009). The percentage of water uptake by the matrices was calculated for each time point, and graphed to illustrate the absorption over the time. The data was analyzed using the first- and second-order kinetic equations. Similar study was performed on ternary isotropic solutions to investigate whether the existence of ethanol would affect the absorption behavior of phytantriol.

2.4. In vitro drug release

The docetaxel-loaded isotropic solutions were prepared by mixing the required amount of docetaxel with ethanol and adding the appropriate amount of phytantriol, and then vortex-mixing homogeneously. After water being added, the mixture was vortex-mixed to homogeneous and then centrifuged at 3000 rpm for 20 min.

The release study was conducted using dialysis membrane tubing (Chang and Bodmeier, 1998). Briefly, the docetaxel-containing isotropic solutions in triplicate were placed separately in dialysis bags (14,000 Da) which inserted into a small purpose-built glass tube (Fong et al., 2009) with approximately 500 μl capacity to ensure a well-defined reproducible surface area (200 mm^2) for docetaxel release. After sealing it, the dialysis bags were taken out of the glass tube and tied to a glass stick, then immersed into 15 ml of pH 7.4 PBS. Besides, it was confirmed that the diffusion of the docetaxel molecules across the membrane was not the rate-limiting step (Yanasarn et al., 2009). At the predetermined release time, the release medium was withdrawn and replaced with the fresh to maintain the sink condition. The medium samples were analyzed for drug content using reverse-phase high-performance liquid chromatography (HPLC), and the release data was analyzed to determine the applicable release model. The influences of docetaxel concentration or the initial compositions of ternary isotropic solutions on the release behavior were investigated.

2.5. HPLC analysis

The amount of docetaxel released was analyzed by HPLC (1200 series, Agilent Technologies, United States) using a C18 column (5 μm, i.d.) at a wavelength of 230 nm. The mobile phase was composed of 70% methanol and 30% deionized water eluted isocratically at a flow rate of 1.0 ml/min. Samples were filtered using 0.2 μm filters, and an autosampler was used to inject 20 μl samples. The method was validated by standard methods for standard curve ($A = 23.551C + 5.6786$, $r = 0.9999$, 0.4–100 μg/ml), precision and accuracy.

2.6. Phase behavior determination

Due to the inherent high viscosity of the cubic liquid crystalline phase, a low viscosity precursor system, which was proposed to rapidly form cubic phase as exposing to body fluids, was prepared to enable dosing via gavage injection. Therefore, it was necessary to identify whether the ternary isotropic solution as a precursor system would transform into the cubic liquid crystalline phase after incubated in the release medium. PLM, SAXS, Raman spectroscopic and rheological measurements were used to confirm this transformation during the release study. PLM and SAXS measurements were performed as described in Section 2.2. Raman spectroscopic measurements were carried out with a laser micro-Raman spectrometer (Renishaw inVia, Britain), the excitation of which was