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Phytantriol-based lyotropic liquid crystal as a transdermal delivery system

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ABSTRACT

The purpose of this study was examined the feasibility of using phytantriol-based cubic and hexagonal liquid crystal preparation for the percutaneous administration of *trans*-cinnamaldehyde (TCA). TCA-loaded lyotropic liquid crystal formulations were prepared and characterized, their skin permeability *in vitro* and *in vivo* was evaluated. Preliminary pharmacodynamics were also investigated in adjuvant arthritics (AA) rats. The formulations were identified respectively as cubic and hexagonal structure. The *in vitro* permeability study exhibited that both cubic and hexagonal liquid crystal improved the cumulative permeation quantity and permeation rates of TCA compared with home-made gel. The results of an *in vivo* transdermal permeability experiment showed that the area under the curve [$AUC_{(0-\infty)}$] of the hexagonal and cubic liquid crystal was 1.62 and 1.53 times higher than that of the gel group, respectively. Preliminary pharmacodynamics studies indicated that the group of high-dose TCA-loaded (200 $mg{\cdot}kg^{-1}$) hexagonal liquid crystal was shown to inhibit the paw swelling of AA rats, improve synovial hyperplasia and inflammatory cell infiltration, and down-regulate the levels of serum interleukin (IL)-1β and tumor necrosis factor (TNF)-α. Furthermore, there was no significant difference in the anti-inflammatory effects of TCA-loaded hexagonal liquid crystal and the commercially available product Voltaren® emulgel®. Thus, hexagonal liquid crystal was considered as an effective delivery system for TCA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease that generally occurs in the hands, wrists, and joints. It is characterized by chronic, symmetrical, and synovial arthritis (Alunno et al., 2017; Grainger and Rowbotham, 2013; Tarner and Müller-Ladner, 2008). Synovitis can cause cartilage damage and bone erosion, resulting in joint deformity or even disability. Currently, there is no specific drug for treatment of RA, and RA treatment mainly entails the control of inflammation, and prevention of articular cartilage damage and loss of joint function (Butoescu et al., 2009; Kumar et al., 2016). *trans*-Cinnamaldehyde (TCA), an olefin aldehyde, is the main component in the volatile oil of Gui Zhi which widely used to treat rheumatoid disease (Huang et al., 2015; Huang et al., 2016; Nozaki et al., 2014), and TCA has anti-inflammatory and analgesic effect (Zhu et al., 2017). It has been reported that TCA has an anti-inflammatory effect by reducing the expression of Toll-like receptor and TRAF6, and inhibiting the nuclear translocation of NF-Kb (Zhao et al., 2015). Moreover, percutaneous delivery enables the elevation of local drug concentrations and extends the time of effective drug concentration, is a suitable administration for RA treatment because RA requires long-term medication to retain a sufficiently effective concentration of drug at the joint lesion site (Dangol et al., 2016; Swain et al., 2011). Percutaneous delivery of TCA should enable a great drug penetration, therefore, the development of suitable delivery system can be a solution.

In recent years, lyotropic liquid crystals (LLCs), formed by the self-assembly of amphiphilic molecules in a solvent (typically water), have gained wide attention due to their excellent drug-loading and drug-release properties (Brown, 1971; Chen et al., 2014; Rajabalaya et al., 2017). LLCs can mainly be classified into lamellar (L_α), cubic (V_2), and hexagonal (H_2) phases according to their different internal structures (Milak and Zimmer, 2015). Among these, the V_2 and H_2 phases are the most commonly used drug carriers. These structures coexist with water, which offers the potential of slow-release matrix for active pharmaceutical ingredients of various sizes and polarities (Drummond and Fong, 1999; Kudla et al., 2010; Lee et al., 2009; Shah et al., 2001). When formulated for transdermal drug delivery, both V_2 and H_2 phases are transparent gels with structures that are similar to biofilm lipid, and

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have been proved capable of promoting drug penetration (Elisabetta et al., 2005; Lara et al., 2005; Lopes et al., 2006; Lopes et al., 2007; Peng et al., 2010; Yongtai et al., 2014).

Glyceryl monooleate (GMO) and phytantriol (PT) (Barauskas and Landh, 2003; Lee et al., 2009; Zabara and Mezzenga, 2014) are lipid materials that are most commonly used for the preparation of lyotropic liquid crystals. GMO and PT have similar behaviors in water. However, while GMO contains ester bonds that can be easily hydrolyzed by esterases, thereby destroys the liquid crystal structure. PT can maintain its liquid crystal structure at room temperature, and even in digestive juice (Avachat and Parpani, 2015). PT can form the V_2 phase in excess water (Barauskas and Landh, 2003). And the V_2 phase can be transformed into the H_2 phase at 80 °C. Nevertheless, transformation of the V_2 phase of PT to the H_2 phase at physiological temperature can be achieved by the addition of a triglyceride (TAG) (Bitancherbakovsky et al., 2014).

In this study, we evaluated the feasibility of using PT-based V_2 and H_2 phases of TCA for percutaneous drug administration treating RA. The formulations were prepared *via* self-emulsification, and the resulting phase structure was confirmed by crossed-polarized light microscopy (CPLM), small-angle X-ray scattering (SAXS), and rheological measurements. The skin penetration of TCA was assessed by *in vitro* percutaneous permeation study using the abdominal skin of rats and *in vivo* pharmacokinetic study. The anti-inflammatory effects of TCA-loaded liquid crystal were studied in adjuvant arthritic (AA) rats, whereas commercially available Voltaren® emulgel® was used as a positive control drug. And as pharmacodynamic indications, we assessed the degree of edema, performed a pathomorphological examination of the synovium, and measured the levels of serum IL-1β and TNF-α.

2. Materials and methods

2.1. Materials

PT (GC > 95%) was purchased from Tokyo Chemical Industry Co., Ltd. (Shanghai, China). TCA (GC > 99%) and TAG (GC > 99%) were obtained from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). Cinnamic acid (GC > 98%), used as a reference substance, was obtained from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Voltaren® emulgel® was purchased from Beijing Novartis Pharmaceutical Co., Ltd. (Beijing, China). Freund's complete adjuvant was purchased from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). Rat interleukin 1β (IL-1β) ELISA kits and tumor necrosis factor-α (TNF-α) ELISA kits were purchased from Beijing Andi Huatai Biological Technology Co., Ltd. Purified water produced using a Milli-Q system (Millipore, Bedford, USA) was used throughout the experiment. All reagents were of analytical grade, except for acetonitrile, methanol, and acetic acid, which were of a chromatographically pure grade.

Wistar rats (250 ± 20 g) were supplied by the Experimental Animal Center of Anhui Medical University (Anhui, China). All animal experiments were conducted in accordance with the guidelines of the Laboratory Animal Center of Anhui University of Chinese Medicine, and all animal protocols were approved by the Animal Ethics committee of Anhui University of Chinese Medicine.

2.2. Preparation of TCA-loaded liquid crystal

2.2.1. Preparation of TCA-loaded V_2 phase

The TCA V_2 phase (F1) used in experiments comprised PT (77 wt%), water (20 wt%), and TCA (3 wt%). Appropriate quantities of PT and TCA were mixed while heating to 60 ± 0.5 °C and then vortex-mixed for 5 min. A specific amount of preheated water at the same temperature was added into the mixture, followed vortex mixing to homogeneity. The preparations were maintained in a closed vial and equilibrated for 1 week at 25 ± 0.5 °C.

2.2.2. Preparation of TCA-loaded H_2 phase

Formulations of the TCA H_2 phase contained PT, TAG, TCA, and water (F2: 73.15:3.85:3:20, w/w/w/w). The preparative method used for the TCA H_2 phase was the same as that used for the TCA V_2 phase, and the formulated amount of TAG was dissolved in the PT-TCA mixture prior to its incorporation into the H_2 phase.

2.3. Characterization of TCA-loaded liquid crystal

2.3.1. CPLM

Formulation samples were observed at room temperature by placing a small amount of sample on a glass slide and viewing under a polarized light microscope (CK-500; Caikon, China). Photographs of the preparation were monitored by computer.

2.3.2. SAXS

SAXS measurements (Anton Paar, Austria) were used to determine the effect of temperature (25 and 32 °C) on the internal structure of TCA V_2 phase (F1) and TCA H_2 phase (F2). The effect of loading dose (0, 1, 2, and 3 wt%) on the lattice parameter of F2 was investigated using SAXS at 25 °C. The sample was placed in a SAXS test chamber under vacuum. After equilibrating for 10 min, scattering experiments were performed using an X-ray source (Cu Kα radiation, 0.154 nm) operated at 40 kV and 50 mA for 10 min. An image board with aluminum foil as the background recorded the scattering message, and the scattering files subsequently subtracted the scattering message of aluminum. Scattering intensities were plotted *versus* the scattering vector (q) and from this plot the lattice parameters were calculated.

2.3.3. Rheological measurements

The rheological properties of LLCs were investigated using a DHR-2 rheometer (TA, America) incorporating a cone-plate sensor with a diameter of 20 mm, a cone angle of 1°, and a gap of 28 μm. An appropriate amount of sample was loaded onto the sample stage, and then the senor was slowly adjusted to obtain the desired measuring gap. The measurement procedure commenced after 2 min of equilibrium at the measured temperature. A viscoelastic test was performed prior to conducting the flow test as the steady-state shear test damages samples. Using the oscillation-amplitude mode and a scanning frequency fixed at 1 Hz, amplitude sweep measurements were conducted in the range of 0.01%–100% strain% to determine the linear viscoelastic region of the sample. Taking a value in the linear viscoelastic region, frequency sweep measurements were conducted in the range of 0.01–100 rad·s^{-1} using the oscillation-frequency mode, with the measurement temperature being maintained at 32 ± 0.1 °C. The rheological properties of TCA V_2 phase (3%) and TCA H_2 phase (3%) were investigated, and the effects of loading dose (0, 1, 2, and 3 wt%) on the relaxation time of TCA H_2 phase were investigated. To investigate the viscosity of TCA-loaded liquid crystal, the shear rate was accelerated from 1 s^{-1} to 500 s^{-1} using the flow-sweep mode in the flow test.

2.4. In vitro percutaneous metabolism and permeation study

2.4.1. Metabolism of TCA in rat skin homogenates

Skin excised from the depilated abdomens of male rats was cut into pieces. A mixture of skin pieces and pre-cooled phosphate buffer solution (PBS, pH 6.8) was homogenized to prepare a 10 wt% rat skin homogenate. The resulting preparation was centrifuged at 3000 rpm for 15 min and the supernatant collected. Homogenates containing TCA at concentrations of 50, 100, and 150 μg·mL^{-1} were prepared using 5 mL of the resulting supernatant, an appropriate amount of TCA, and 15 mL of PBS. For the controls, PBS was used instead of the supernatant to prepare TCA solutions of the same concentrations. At 0, 1, 3, 6, 9, 12, and 24 h intervals, 1 mL of the solution was withdrawn and added to the same volume of acetonitrile. The mixtures were centrifuged at 3000 rpm for 10 min, filtered through 0.45-μm membranes, and the