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H_{II} mesophase as a drug delivery system for topical application of methyl salicylate

Xin Liang [a], Yu-lin Chen [a], Xiao-jing Jiang [a], Sheng-mei Wang [a], Ji-wen Zhang [b], Shuang-ying Gui [a,c,d,*]

[a] Department of Pharmacy, Anhui University of Chinese Medicine, Hefei, Anhui 230012, China
[b] Center for Drug Delivery System, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China
[c] Institute of Pharmaceutics, Anhui Academy of Chinese Medicine, Hefei, Anhui 230012, China
[d] Key Laboratory of Xin'an Medicine, Ministry of Education, Hefei, Anhui 230012, China

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ABSTRACT

The main objective of this study was to develop reversed hexagonal (H_{II}) mesophase for transdermal delivery of methyl salicylate. The formulation was prepared, characterized and evaluated for its skin penetration in vitro and skin retention in vivo. Preliminary pharmacodynamics and skin irritation were also investigated. The formulation was identified as hexagonal structure. In vitro study exhibited that H_{II} mesophase enhanced the skin permeation by delivering 2.61 times more methyl salicylate than the commercially available cream. Meanwhile, H_{II} mesophase presented higher bioavailability as $AUC_{(0-24)}$ and $AUC_{(0-\infty)}$ were 32.894 $\mu g \cdot mL^{-1}$ and 32.935 $\mu g \cdot mL^{-1}$ respectively, while the cream were 12.791 $\mu g \cdot mL^{-1}$ and 12.970 $\mu g \cdot mL^{-1}$. Preliminary pharmacodynamics studies demonstrated that H_{II} mesophase possessed anti-inflammatory and analgesic effects for inhibiting paw edema, granuloma and pain. MeSa H_{II} mesophase showed no skin irritation on the normal rat skin. Thus, H_{II} mesophase was considered as an effective delivery system for MeSa.

1. Introduction

Methyl salicylate (Methyl 2-hydroxybenzoate, MeSa), extracted from wintergreen, a kind of traditional Chinese herbal medicine, is recognized as an anti-inflammatory and analgesic agent. By producing vasodilatory results, MeSa increases localized blood flow and tissue temperature (Green and Flammer, 1989). As a common type of pain relievers, MeSa has been used for the treatment of musculoskeletal pain like swelling, strain or twist. Because MeSa has an irritating effect on gastrointestinal mucosa, it is available over the counter only for external use. Dosage formulations, including creams, lotions, gels, and topical patch products, have shown efficient therapies (Higashi et al., 2010). However, according to FDA drug safety podcast, some formulations of MeSa were reported causing serious skin burns (http://www.fda.gov/drugs/drugsafety/drugsafetypodcasts/ucm319512.htm, 2012). The vasodilatory results of MeSa may have a risk of causing skin irritation. In addition, when delivered into skin, MeSa is prone to decompose into salicylate acid (2-Hydroxybenzoic acid), which leads to poor bioavailability (Shen et al., 2001). Percutaneous delivery of MeSa should enable an effective drug concentration for achievement of anti-inflammatory and analgesic effect. Therefore, the development of suitable delivery system can be a solution to overcome the disadvantages.

Liquid crystalline is a type of self-assembled structure of amphiphile molecules in solvent and has great structural versatility (Mulet et al., 2013; Akhlaghi et al., 2016). The common phase can be identified as: lamellar, cubic and hexagonal phase. Reversed hexagonal (H_{II}) mesophase is comprised of dense packed, infinitely long and straight water-filled rods. These rods arrange in hexagonal lattice and are separated by lipid bilayers (Amar-Yuli and Garti, 2005; Libster et al., 2009a). H_{II} mesophase can accommodate hydrophilic drugs in the water channels, locate lipophilic drugs within the lipid hydrophobic moieties and position amphiphilic drugs in the interface (Amar-Yuli and Garti, 2005; Amar-Yuli et al., 2008; Libster et al., 2009b). H_{II} mesophase is bioadhesive, biodegradable and physically stable. Its rheological properties indicate it is tailored to develop gel-like formulation (Geraghty et al., 1997; Yamada et al., 2011). It has the potential for being developed as transdermal delivery system. H_{II} mesophase presents similar structural and chemical characteristics of living cell membranes with high mutual affinity, providing the possibility to mimic the penetrating mechanism and perforate cell membranes (Cohen-Avrahami et al., 2010). H_{II} mesophase exhibited increased skin penetration of lipophilic compounds when used as transdermal delivery (Lopes et al., 2007;

* Corresponding author at: Department of Pharmacy, Anhui University of Chinese Medicine, No.1, Qianjiang Road, Hefei, Anhui 230012, China.
E-mail address: guishy0520@126.com (S. Gui).

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Libster et al., 2009a). The increased penetration mainly observed in the epidermis and dermis (Lopes et al., 2006). It has been proved that H_{II} mesophase could work as a protect carrier to improve drug's stability against denaturation (Libster et al., 2011; Amar-Yuli et al., 2011). Moreover, the preparation of H_{II} mesophase is simple and energy-saving. Due to these properties, H_{II} mesophase can be specifically used for transdermal delivery system.

Phytantriol (3,7,11,15-tetramethylhexadecane-1,2,3-triol, PT) is a lipid commonly utilized in cosmetic preparations of hair and skin care. It works as a penetration promoter and improves the moisture retention capacity (Erlemann et al., 1994). Recently, PT is employed as self-assembling aggregates. As a kind of small non-ionic amphiphile, PT is capable of swelling in the water and could form well-ordered liquid crystalline phases spontaneously (Barauskas and Landh, 2003; Lee et al., 2009). The main materials for preparing liquid crystalline include glyceryl monooleate (GMO) and PT. The ester-based structure of GMO may limit its practical application. The ester hydrolysis may lead to chemical instability and disruption of liquid crystalline structure (Dong et al., 2006). While PT comprises a highly branched phytanyl tail with a trihydroxy headgroup, and importantly, its structure does not include ester linkages and possesses complete saturation of aliphatic chain. It has relatively high resistance to hydrolysis. Thus, PT is more stable, compared to GMO (Qin et al., 2015).

In our previous study, MeSa was incorporated into PT-based cubic mesophase. The increase of drug loading induced a cubic-to-hexagonal phase transition. The mesophase presented H_{II} mesophase when the drug loading was over 8% MeSa (Wu et al., 2015). In present work, PT-based H_{II} mesophase of MeSa was investigated. The formulation was prepared. The structure of mesophase was confirmed by crossed-polarized light microscopy (CPLM), small angle X-ray scattering (SAXS) and rheological measurements. The skin penetration of MeSa was assessed in vitro using mice skin in a Franz diffusion cell. The skin retention test was performed in vivo on mice by pharmacokinetics of local skin. Paw edema test, cotton pellet-induced granuloma test and hot plate test were employed to demonstrate anti-inflammatory and analgesic effects for preliminary pharmacodynamics study. Skin irritation study was also carried out on rats with normal skin and damaged skin.

2. Materials and Methods

2.1. Materials

PT (GC > 95%) was purchased from Tokyo Chemical Industry Co., Ltd. (Shanghai, China). MeSa (GC > 95%) was obtained from Aladdin Industrial Co. (Shanghai, China). Purified water from a Milli-Q system (Millipore, Bedford, USA) was used throughout the experiment. Compound MeSa cream (Mentholatum, (China) Pharmaceuticals Co., containing 12.7%MeSa).

2.2. Preparation of MeSa H_{II} Mesophase

The composition of MeSa H_{II} mesophase used in the experiments was 66 wt% PT, 12 wt% water and 22 wt% MeSa. Weighted quantities of MeSa and PT were mixed while heating to 60 ± 0.5 °C. The mixture was stirred homogeneously for 3 min and ultrasonic mixed for 10 min. The appropriate quantity of preheated water at the same temperature was added into the mixture and vortex-mixed for 5 min. The resulting formulation was kept in a closed vial at room temperature one week for equilibration. The formulation presented transparent gel-like liquid crystalline.

2.3. Characterization of MeSa H_{II} Mesophase

2.3.1. CPLM

The equilibrated sample was thinly spread on the glass slide. The cover glass was placed slowly on the glass slide so as not to form air bubbles. CPLM (CK-500, Caikon, China), equipped with pc monitor, was used to identify the phase structure of the samples. The photographs were taken under the condition of the cross-polarizer.

2.3.2. SAXS Measurement

The internal structure of liquid crystalline can be identified by SAXS (Yamashita et al., 2007; Nilsson and Soderman, 1996). The measurements were performed on an SAXS mc^2 (Anton Paar, Austria) with X-ray source of wavelength $\lambda = 0.154$ nm and operated at 40 kV and 50 mA. The optics and sample chamber were under vacuum to minimize air scatter. Measurements were performed at 37 °C, and samples were equilibrated for 10 min prior to collection of scattering pattern. Scattering files were background subtracted. Scattering intensities were plotted versus q-value, which enabled the identification of peak positions.

2.3.3. Rheological Measurement

Rheological measurements were carried out with a rheometer (DHR2, TA, America). A cone-plate sensor was used with a diameter of 40 mm, cone angle of 1°, and a gap of 28 μm. The measuring temperature was maintained at 32 °C. The sample was gently placed onto the top of the plate, and then the sensor was slowly lowered to its measuring position with constant velocity. Any excess sample squeezed out from the sensor system was gently removed. Frequency sweep measurement was performed at a constant stress which was in the linear viscoelastic region. The sample was exposed to a gradual increase of frequency of 0.01 to 1000 Hz. The storage modulus (G′) and the loss modulus (G″) were used to construct the curves for characterizing the viscoelasticity.

2.4. In Vitro Percutaneous Permeation Study

To assess whether the H_{II} mesophase could facilitate skin penetration of MeSa, Franz diffusion cell system and abdominal skin of male mice were used. The mice skin was excised from depilated abdomens of Wistar mice. The subcutaneous fat was carefully removed. The skin samples were cleaned in normal saline, stored at −20 °C, and used within a month. On the day of the experiment, the skin was thawed and mounted in a Franz diffusion cell (diffusion area of 3.14 cm^2), with the stratum corneum (SC) facing the donor compartment (where the formulation was applied) and the dermis facing the receptor compartment. Phosphate buffer solution (PBS, pH 6.8) was employed as receptor solution to mimic the skin environment. The receptor compartment was filled with 8.0 mL receptor solution to maintain sink conditions. The receptor solution was kept at 32 ± 0.5 °C with a stirring rate of 120 rpm. 0.5 g of 12% MeSa H_{II} mesophase was applied on the surface of the SC. At the time intervals of 1,2,4,6,10,12,24 and 48 h, 1 mL of receptor fluid was withdrawn and replaced with an equivalent volume of preheated PBS (pH 6.8) immediately. For the control, 0.47 g of compound MeSa cream was spread on the other group of rat skin. The release behavior was investigated in the same manner. The diffusion test was performed in triplicate for each formulation. The receptor fluid samples were filtered through 0.45 μm membranes and quantified by high performance liquid chromatography (HPLC). Both MeSa and salicylate acid were determined. Cumulative amount of drug released were calculated and plotted against time.

2.5. In Vivo Skin Retention Study

63 male Wistar mice (20 ± 2 g, 2–3 month) in total were used for the experiment. The animals were provided with standard food and water ad libitum. They were exposed to a daily 12:12-h light:dark cycle a week for acclimatizing to laboratory conditions. On the day of the experiment, 3 of them were picked randomly as the baseline group to estimate normal physiological parameters. The remaining mice were assigned randomly to 2 groups (n = 30). The group was