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Research paper

Improved transdermal delivery of morin efficiently inhibits allergic contact dermatitis

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ABSTRACT

The skin is an important site for local or systemic application of drugs. However, most of the drugs have poor permeability through the skin's outermost layer, stratum corneum. The aim of this study was to develop a method to enable transdermal delivery of morin (3, 5, 7, 2, 4-pentahydroxyflavone), which is a poorly water-soluble drug with anti-inflammatory properties obtained from natural products. Morin phospholipid complex (MPC) was prepared and then loaded in Carbopol 940 hydrogel (MPC-gel), which can significantly increase the transdermal flux of morin based on the in vitro skin penetration data presented in this paper. To further enhance permeation, different compositions of penetration enhancers were dispersed in the gel and screened. After applied onto the mouse skin, MPC-gel showed apparent reduction of ear swelling in 2, 4-dinitrofluorobenzene (DNFB)-induced allergic contact dermatitis (ACD). Further determination of cytokines levels, histopathological analysis and T lymphocytes proliferation indicates that the MPC-gel is potent enough to reduce the inflammatory response mediated by the DNFB in ACD mice model. Collectively, we anticipate that such an approach may provide a new treatment for topical ACD.

1. Introduction

Stratum corneum (SC), the uppermost layer of the skin, is a challenge for transdermal drug delivery and is the main physical barrier to drug permeation (Vitorino et al., 2013). Over the past decade, successful attempts to solve this problem have been made through chemical permeation enhancers, microneedles, elctroporation, ultrasound and low-frequency ionophoresis, but success has been limited (Han and Das, 2013; Lane, 2013; Tuan-Mahmood et al., 2013). The potential for skin irritation associated with chemical permeation enhancers and the invasiness of microneedles, and ease of application of some electrical devices all have their disadvantages with respect to transdermal delivery. The complexity and expensive cost of production of many transdermal drug delivery systems may also deter clinical applications of these systems. The use of chemical penetration enhancers (CPEs) may be considered as a relatively more cost-effective approach. These CPEs have been used in transdermal research since the 1960s (Aqil et al., 2007; Barry, 2004). Terpenes, such as menthol, cineole and limonene, obtained from natural sources are generally considered as safe and effective penetration enhancers, where the Food and Drug Administration classifies terpenes as generally regarded as safe (GRAS) (Vaddi et al., 2002).

Allergic contact dermatitis (ACD) is a common chronic skin inflammatory disorder caused by T cell-mediated delayed hypersensitivity of type IV, which has a significant impact on patients' quality of life (Fonacier et al., 2015; Kaplan et al., 2012). Approximately, 15–20% of the world' s population suffer from this condition (Keegel et al., 2009; Shi et al., 2015). Haptens, such as 2, 4-dinitrofluorobenzene (DNFB) could easily penetrate into the skin. Dendritic cell (DC) maturation is induced after the haptens entry into the epithelia and covalently bind to carrier proteins to cause a full immunogenic response (Kadowaki, 2007). Therefore, in vivo models of ACD are often used to investigate Ag-specific T cell-mediated immune responses (Saary et al., 2005). Apart from ACD, T cells also play a crucial role in many other autoimmune-related diseases such as systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis (Petta et al., 2014). Glucocorticoid

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is one of the most commonly used anti-inflammatory drugs clinically, but its systemic use often results in severe side-effects particularly after prolonged use and or high doses, due to the ubiquitous glucocorticoid receptor distributed throughout the body (Baschant et al., 2012). Thus, alternative treatments that avoid the need for glucocorticoid whereby the toxicity and side-effects are minimized are desirable.

Recently, several effective flavonoid compounds such as morin hydrate (3, 5, 7, 2, 4-pentahydroxyflavone) and quercetin have been found in fruits, wine, tea, and other family members of herbs (Das, 1994). These low molecular weight substances have shown to possess anti-inflammatory, anti-oxidative, anti-cancer, and anti-hypertensive properties, where they have helped to improve eye-sight and strengthen joints (Fang et al., 2003; Francis et al., 1989; Iwase et al., 2001; Kang et al., 2004; Kitagawa et al., 2004). The main advantage of flavonoids over synthetic immunomodulatory compounds is that they are relatively non-toxic and can be used as a dietary supplement (Jakhar et al., 2014; Penalva et al., 2016). In some countries, quercetin is accepted as a dietary supplement with the recommended daily oral doses of 200–1200 mg (Penalva et al., 2017). However, only a few studies have focused on the applicable formulation of morin. Most investigations employ simple aqueous formulations of morin and administering high doses via the intraperitoneal injection route to achieve the effective pharmacological effect (Sinha et al., 2015; Zhang et al., 2010). Injection administration provides effective systemic treatment but need for frequent application, skilled administration and potential for abuse warrant other means of delivery (Luby et al., 2005). Thus, the potential of developing a transdermal form of morin to help improve patient compliance-by providing a sustained delivery of low therapeutic doses of the drug transdermally was investigated. As a poorly water-soluble drug, the use of phospholipid complex has been studied in the literature to help improve the solubility of morin for oral application, and the researchers found that morin phospholipid complex (MPC) can significantly improve both the lipophilicity and hydrophilicity of morin (Zhang et al., 2015, 2011). However, to date, the therapeutic effect of morin via transdermal delivery has not been fully established.

Carbopols are hydrophilic polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol, which make them as potential candidates for topical use gel type formulation. They are considered to be non-toxic and non-irritant materials with no evidence of their hypersensitivity on human when used topically (Chawla and Saraf, 2012). Carbopol 940 was selected as a gelling agent because it can produce an acceptable physical appearance, with suitable spreadability due to its water absorption capacity, hydration and swelling ability. In addition, Carbopol 940 has high molecular weight (104,400 g/mol), so they cannot penetrate into the skin and offer good alternative to oil based vehicles (Chawla and Saraf, 2012). In this study, a Carbopol 940 gel based on the use of MPC technique and CPEs to enhance the transdermal delivery of morin and reduce the inflammatory response mediated by the DNFB has been developed.

2. Materials and methods

2.1. Materials

Morin hydrate, ConA, and 2, 4-dinitrofluorobenzene (DNFB) were purchased from Sigma-Aldrich Co. (Shanghai, China). Carbopol 940 was obtained from Yuan Cheng Technology Co. (Wuhan, China). Lipoid E80 (Purified Ovolecithin) was purchased from Shanghai Tai-wei Pharmaceutical Co., Ltd. (Shanghai, China). Menthol, olive oil, isopropyl myristate, and 1, 2-propanediol were obtained from Aladdin (Shanghai, China). Compound dexamethasone acetate cream was purchased from Baiyunshan Pharmaceutical Co., Ltd. (Guangzhou, China). The Mouse ELISA Ready-SET-Go! Kits were purchased from eBioscience (Affymetrix Inc, US). All other chemical reagents were of analytical or high performance liquid chromatography grade.

2.2. Animals

Female BALB/c mice 6–8weeks old were purchased from the Dashuo Experimental Animal Co., Ltd (Chengdu, P. R. China). All mice experiments were approved by the Institutional Animal Care and Use Committee of Sichuan University. Mice were acclimated to the environment 2–7 days, with water and standard diet available ad libitum.

2.3. Preparation of morin-phospholipid complex gel (MPC-gel)

MPC was prepared and characterized according to previous work (Zhang et al., 2011). In order to obtain suitable viscosity values for transdermal application, MPC was loaded into Carbopol 940 hydrogel. Briefly, 160 mg of MPC and prescription amount of chemical penetration enhancers were dispersed in 2.4 ml of ethanol at room temperature by gentle agitation. Then distilled water was added to the ethanol dispersion with a final ethanol concentration of 30% (v/v), and a clear mixture was obtained. Next, 0.5% (w/v) of Carbopol 940 was sub-divided into the mixture under gentle stirring. After Carbopol 940 was fully swollen overnight at room temperature, it was subsequently neutralized using triethanolamine to promote gelation. As a control, Morin-gel was prepared using the process described for the MPC-gel.

2.4. Evaluation of MPC-gel

The prepared MPC-gel was evaluated for drug uniformity, drug content, viscosity, pH, and centrifugation stability studies.

The drug content was determined by taking 1 g of accurately weighed MPC-gel which was diluted with methanol to 100 ml and analyzed by HPLC. The HPLC method we used to determine morin was according to a published method with some modifications (Zhang et al., 2011). The analysis was performed on an Agilent 1260 infinity HPLC system equipped with a G1314C UV detector. A Kromasil C18 (Scienhome, China) analytical column (150 mm × 4.6 mm, 5 μm) was used with a mobile phase consisting of acetonitrile and 0.5% phosphoric acid (35:65, v/v) by a flow rate of 1.0 ml/min. Detection wavelength was 252 nm, and the injection volume was 10 μl. Studies showed that the precision, accuracy, and recovery of this HPLC method all met the measurement requirements.

Viscosity studies were carried out using a DV-C digital viscometer (Brookfield engineering, MA, USA). Briefly, Take the appropriate volume of MPC-gel in a 25 ml beaker with spindle S64 at 4 rpm and at temperature 25 ± 0.5 °C.

The pH of the dispersion was measured using a pH meter (METTLER TOLEDO). Briefly, 1 g of the MPC-gel was weighed in 25 ml volumetric flask and then added distilled water to 25 ml.

As for centrifugation stability studies, MPC-gel was weighed 5 g in 10 ml EP tube, and then centrifuged at 4000 rpm/min for 30 min. Changes in preparation before and after centrifugation were observed. All measurements were done in triplicate.

2.5. In vitro release studies

To identify the characteristics of morin release from Carbopol 940 gel, release studies were carried out based on a dialysis technique. Briefly, 1 g of Morin-gel or MPC-gel was transferred into dialysis bags (MWCO ~ 14,000) and the bags were ligated with surgical suture lines. Then, the sealed bags were dialyzed against 50 ml of release medium (PBS, pH 7.4) and shaken in a horizontal