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Co-delivery of evodiamine and rutaecarpine in a microemulsion-based hyaluronic acid hydrogel for enhanced analgesic effects on mouse pain models

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ABSTRACT

The aim of this study was to improve the analgesic effect of evodiamine and rutaecarpine, using a microemulsion-based hydrogel (ME-Gel) as the transdermal co-delivery vehicle, and to assess hyaluronic acid as a hydrogel matrix for microemulsion entrapment. A microemulsion was formulated with ethyl oleate as the oil core to improve the solubility of the alkaloids and was loaded into a hyaluronic acid-structured hydrogel. Permeation-enhancing effects of the microemulsion enabled evodiamine and rutaecarpine in ME-Gel to achieve 2.60- and 2.59-fold higher transdermal fluxes compared with hydrogel control ($p < 0.01$). The hyaluronic acid hydrogel-containing microemulsion exhibited good skin biocompatibility, whereas effective ME-Gel co-delivery of evodiamine and rutaecarpine through the skin enhanced the analgesic effect in mouse pain models compared with hydrogel. Notably, evodiamine and rutaecarpine administered using ME-Gel effectively down-regulated serum levels of prostaglandin E_2, interleukin 6, and tumor necrosis factor α in formaldehyde-induced mouse pain models, possibly reflecting the improved transdermal permeability of ME-Gel co-delivered evodiamine and rutaecarpine, particularly with hyaluronic acid as the hydrogel matrix.

1. Introduction

Evodiamine (Evo) and rutaecarpine (Rut) are major bioactive alkaloids isolated from the traditional Chinese medicine Wu-Zhu-Yu (Fructus Evodiae), which is the dried fruit of *Evodia rutaecarpa* (Juss.) Benth (Fig. 1) (Chinese Pharmacopoeia Committee, 2015). Evo and Rut show anti-inflammatory and anti-nociceptive effects in various pain models and exhibit anti-inflammatory activity after transdermal application (Choi et al., 2006; Ko et al., 2007; Shin et al., 2007; Yarosh et al., 2006). Transdermal administration minimizes hepatic first-pass metabolism, avoids bioavailability problems, and increases patient compliance, compared to oral and parenteral routes (Delgado-Charro and Guy, 2014; Lasagna and Greenblatt, 1986). However, Evo and Rut are insoluble in water, which may reduce *in vivo* absorption and decrease bioavailability.

As a promising vehicle for transdermal drug delivery, microemulsions (MEs), nanocarriers with the particle size of 10–100 nm, have received increasing attention in recent years (Cavalcanti et al., 2016). Defined as dispersions consisting of oil, surfactant, cosurfactant, and aqueous phase, microemulsion systems have several advantages, including ease of manufacturing, increased drug solubility, good thermodynamic stability, and enhanced drug permeation compared to conventional formulations (Mouri et al., 2016). Microemulsions allow sustained or controlled drug release after transdermal administration and have been used as transdermal delivery vehicles for many drugs (Goindi et al., 2015; Todosijević et al., 2015; Zhai Y and Zhai G, 2014).

In previous studies, we successfully prepared a microemulsion-based transdermal delivery system for co-delivery of Evo and Rut and demonstrated that this optimized nanoscale vehicle formulation significantly enhanced the permeation of Evo and Rut, compared to conventional preparations (Zhang et al., 2011). However, the fluidity of ME poses an inconvenience for patients. Hydrogels are generally used as vehicles for ME droplets; however,

Abbreviations: DLS, dynamic light scattering; EE, entrapment efficiency; ELISA, enzyme-linked immunosorbent assay; Evo, evodiamine; Gel, hydrogel; HA, hyaluronic acid; H&E, hematoxylin and eosin; IL-6, interleukin 6; ME, microemulsions; ME-Gel, microemulsion-based hydrogel; PGE_2, prostaglandin E_2; Rut, rutaecarpine; TEM, transmission electron microscopy; TNF-α, tumor necrosis factor α; AAIPM, acetic acid; FIPM, formaldehyde; DIPM, dimethylbenzene; HWPM, hot water–induced pain models.

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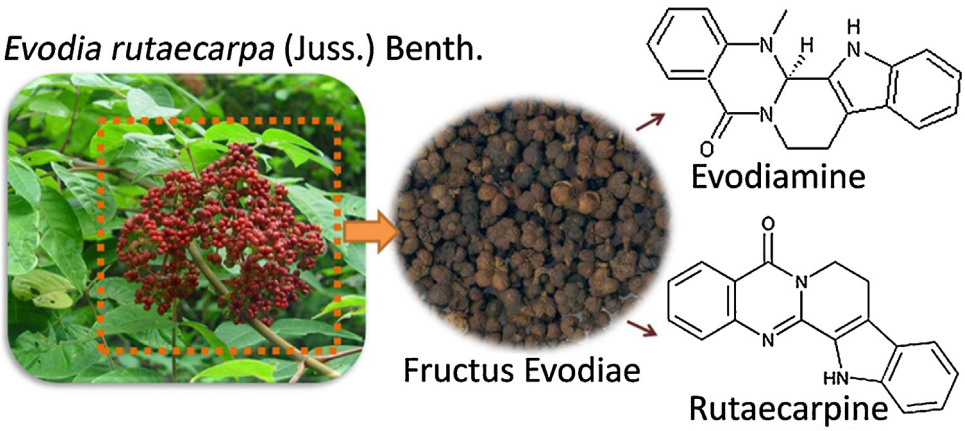

Fig. 1. Evodiamine and rutaecarpine sources and chemical structures.

some hydrogel matrices may destabilize the ME, as seen with Carbomer-based hydrogel using triethanolamine as a neutralizer (Djekic et al., 2015, 2016; Wan et al., 2015). Hyaluronic acid (HA) is an excellent biomaterial mostly used in medicine and cosmetic industry (El-Dakdouki et al., 2013). As a ubiquitous carbohydrate polymer present in the extracellular matrix, HA is characterized by outstanding biocompatibility compared to synthetic polymers (Mokhtarzadeh et al., 2017). Hydrogel formed by HA adheres well to the skin surface and is an efficient moisturizer, enhancing skin hydration and improving drug permeation (Salwowska et al., 2016). Notably, the hydrogel can be structured with only water, which is greatly beneficial for avoiding demulsification of cargo ME (Alkrad et al., 2016).

In this study, HA was chosen to formulate microemulsion-based hydrogel (ME-Gel) for co-delivery of Evo and Rut, in order to enhance drug permeation after topical application and improve patient compliance. Analgesic effect of Evo and Rut co-delivered using this combinational vehicle were evaluated in four mouse pain models.

2. Material and methods

2.1. Materials

Evo and Rut (purity 98%) were provided by Linuo Biotechnology Co, Ltd (Zhengzhou, China). Ethyl oleate was purchased from Shanghai Yunhong Chemical Preparation Auxiliary Technology Co, Ltd (Shanghai, China). Polyethylene glycol (PEG)-35 castor oil (Cremophor® EL) was obtained from BASF (Ludwigshafen, Germany), whereas HA (molecular weight: 200–400 kD) was purchased from FREDA (Ji'nan, China). ELISA kits were obtained from USEN Biotech Co., Ltd. (Shanghai, China) and all other chemicals were purchased from Sinopharm Chemical Reagent Co, Ltd (Shanghai, China), and were of HPLC or analytical grade.

2.2. Animals

Guinea pigs (300 ± 20 g) and Konmin mice (20 ± 2 g) were used in the study. All animal experiments were conducted with the approval of the Animal Ethical Committee, Shanghai University of Traditional Chinese Medicine. The animals were kept in a suitable environment with free access to food and water and were acclimatized for at least 1 week before the start of the study.

2.3. Preparation of Evo and Rut-loaded ME, ME-Gel, and Gel

Compositions of the prepared formulations are listed in Table 1. The ME formulation was optimized in our previous studies (Zhang et al., 2011). For the preparation of ME, Evo and Rut were added to a mixture of ethyl oleate, Cremophor EL, and PEG 400, stirred at 300 rpm for about 1 h at room temperature with a magnetic stirrer to dissolve the drugs, before adding water dropwise to form the ME. ME-Gel and hydrogel (Gel) were made by adding HA into ME or water and stirring for 12 h at 25 °C.

2.4. HPLC assay

The LC-2010A HT Liquid Chromatograph system (Shimadzu Corporation, Kyoto, Japan) was used to determine the concentration of Evo and Rut, with a Diamonsil C18 reverse phase column (5 μm, 4.6 mm inner diameter × 25 cm; Dikma Technologies, Inc, Beijing, China). The mobile phase was acetonitrile:water (43:57, v/v) containing 0.04% (w/v) sodium 1-octanesulfonate with a flow of 1 mL/min. The column temperature was 35 °C and the detection wavelength was 225 nm. Percentage recoveries were 96.6-103.4%. Intra-day relative standard deviation values were 1.52% for Evo and 0.97% for Rut, whereas inter-day relative standard deviation values were 2.06 and 1.91% for Evo and Rut, respectively.

Table 1
Composition and characteristics of the prepared Evo and Rut-loaded formulations (for droplet size and EE detection of ME, n = 3).

Formulations	CEL (%, w/w)	PEG 400 (%, w/w)	EO (%, w/w)	HA (%, w/w)	Drug concentration (‰, w/w)		Mean size (nm)	EE %	
					Evo	Rut		Evo	Rut
ME	30	15	5	–	2	2	71.5 ± 3.1	98.6 ± 11.4	96.2 ± 8.7
ME-Gel	30	15	5	2	2	2	–		
Gel	–	–	–	2	2	2	–		

Evo, evodiamine; Rut, rutaecarpine; CEL, Cremophor EL; EO, ethyl oleate; HA, hyaluronicacid; EE, entrapment efficiency.