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Research Paper

Ionic liquid – microemulsions assisting in the transdermal delivery of Dencichine: Preparation, *in-vitro* and *in-vivo* evaluations, and investigation of the permeation mechanism

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ABSTRACT

A novel microemulsion was developed and characterized for topical delivery of Dencichine (Den). Two imidazaolium ionic liquid, 1-hydroxyethyl-3-methylimidazolium chloride ([HOEIM]Cl) and 1-butyl-3-methylimidazolium dodecanesulfate ([BMIM]$C_{12}SO_3$) were incorporated into the aqueous and surfactant phases respectively for the remarkable enhancement on skin permeation. The nano-carrier was developed and optimized based on a pseudo-ternary phase diagram. The optimized formulation was composed of 50% water/[HOEIM]Cl mix (1:1) as water phase, 20% Tween 80/[BMIM]$C_{12}SO_3$ mix (1:1) as surfactant, 10% propylene glycol as co-surfactant and 20% IPM as oil phase. The o/w microemulsion was then characterized for droplets sizes (47.7 ± 1.5 nm), zeta potential (−14.83 ± 3.64 mV), viscosity (31 ± 4 mPa) and pH (6.71 ± 0.04). *In-vitro* skin permeation assay suggested the strong enhancement of ILs formulation on the topical delivery of Den, which was approximately 10-fold that of the drug aqueous solution. It was found that the nano-carrier can reduce the skin barrier properties by disrupting the regular and compact arrangements of corneocytes, and moderating the surface properties of the stratum corneum, as evidenced by Transdermal Water Loss Evaluation (TEWL), Differential Scanning Calorimetery (DSC) and attenuated total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR). Furthermore, the *in-vivo* pharmacodynamic evaluation indicated the significant hemostatic activity of Den by the topical application of the vehicle. Additionally, the formulation showed minor cell toxicity and skin irritation. Therefore, our work suggested that the ionic liquid microemulsion can be a promising nano-scale vehicle for the topical application of Den to produce desirable pharmacological effects.

1. Introduction

Panax notoginseng (Burk) F.H. Chen, namely Sanqi, is a famous traditional herb medicine in China for the extensively application in facilitating hemostasis, promotion blood circulation and alleviating pain (Chinese Pharmacopoeia, 2015). Dencichine (β-*N*-oxalyl-L-α, β-diaminopropionic acid, Den), is a nonprotein amino acid in *P*. notoginseng, which has been widely reported for its significant hemostatic properties *in-vivo*. Den was found to exert its hemostatic activities in a paracrine fashion, by increasing intracellular calcium, reducting cAMP, and releasing TXA_2 after binding to the AMPA receptors, which resulted in platelet aggregation (Huang et al., 2014). This compound showed potential for clinical usage for diseases characterized by hemorrhage (Liu et al., 2016). However, further applications were limited by the low bioavailability, along with some side effects. Following oral administrations in rats, Den was determined to undergo an intense first pass metabolism, which generated various metabolites, and resulted in a low bioavailability (approximately 10%), and a short half-life (approximately one hour) (Qian., 2012). Furthermore, under high dosages of Den, several neurotoxic symptoms including astasia, head retraction, and neck stiffness, were observed, which were related to the neurological disorders (Rao., 2011). Therefore, transdermal administration could be an alternative way for Den to improve its bioavailability, and also reduce the side effects.

The percutaneous absorption profiles of Den were investigated in our previous work (Li et al., 2015). We found that Den was water soluble, with a low Log *P* value (approximately −2). Due to its hydrophilic nature, the compound exhibited a poor permeability in the lipophilic membrane of the stratum corneum (SC), which led to a low efficiency in transdermal delivery. Additionally, commonly used permeation enhancers, such as *N*-methyl-2-pyrrolidone, menthol, and azone, showed insignificant improvements in the Den percutaneous

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absorption, with *ER* values ranging from 1.1 to 1.5 (Li et al., 2015). Therefore, acceleration and promotion methods for the Den skin permeation were needed to produce desired pharmacological effects.

Ionic liquids (ILs) based microemulsions (MEs) are quite fascinating and interesting research field for scientists. ILs plays a versatile character in the system and making it very much promising for applications. The formation and characterization were developed and enriched by Gao and Sarkar groups respectively. (Kuchlyan et al., 2016; Gao et al., 2009a,b). Practically, as one of the innovative transdermal drug delivery systems, ILs-MEs coupled with the individual advantages of ILs and MEs s (Goindi et al., 2015; Moniruzzaman et al., 2010). ILs can work as one component of a water phase or oil phases, and show excellent solubilizing ability for hydrophobic and hydrophilic drugs (Dobler et al., 2013). The ILs-MEs are able to overcome the inabilities of conventional MEs to dissolve a number of chemicals which are water insoluble, and result in better pharmacological activities of the drugs. Most importantly, ILs can serve as permeation enhancers. In our previous study, 20 imidazaolium ILs had demonstrated skin permeation enhancements for Testosterone (Zhang et al., 2006). Other reports also reported the promotion effects of some ILs on drug transdermal delivery such as 1,4-diazabicyclo[2.2.2]octane and so on (Zakrewsky et al., 2014; Monti et al., 2017). Therefore, ILs-MEs have shown advantages over MEs, in both drug solubilization and percutaneous absorption.

The aim of the present study was the development of an imidazaolium ILs-ME system to assist in the topical delivery of Den. Based upon our previous work (Zhang et al., 2006), 14 imidazaolium ILs were evaluated and screened for their promotional effects on Den permeation. A pseudo-ternary phase diagram was applied to build the ideal o/w ME composition. Then, the prepared formulation was characterized for particle size, zeta potential, transmission electron microscopy (TEM), pH, and viscosity. The *in vitro* permeation and *in-vivo* pharmacodynamic studies of Den were evaluated. A histopathological study was performed to assess the dermal safety of the prepared formulations. Furthermore, the influences of the ILs and ME on the skin barrier properties and SC structures were investigated in order to elucidate the transdermal mechanism of the formulations.

2. Methods and materials

2.1. Chemicals

Dencichine (> 99.5%) was a gift from Kunming Shenghuo Pharmaceutical Group Co.,Ltd (Yunnan, China). 14 imidazaolium ionic liquids (Table 1), namely *N*-methyl imidazolium chloride ([MIm]Cl), *N*-ethyl imidazolium chloride ([EIm]Cl), *N*-butyl imidazolium chloride ([BIm]Cl), *N*-octylimidazolium chloride ([OIm]Cl), 1-butyl-2,3-dimethylimidazolium chloride([BMMIM]Cl), *N*-ethyl imidazolium chloride ([EIm]Cl), 1-butyl-3- methyl imidazolium chloride ([BMIm]Cl), 1-octyl-3-methyl imidazolium chloride ([OMIm]Cl), 1-carboxyethyl-3-methylimidazolium chloride ([HOOCEIM]Cl), 1-hydroxyethyl-3-methylimidazolium chloride ([HOEIM]Cl),1-hydroyethyl-3-methylimidazolium hexafluorophosphate ([HOEIM]PF_6), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM]PF_6), 1-butyl-3-methylimidazolium dodecanesulfate ([BMIM]$C_{12}SO_3$) and 1-butyl-3-methy dihydrogen phosphate ([BMIm]H_2PO_4) were supplied by Lanzhou Institute of Chemical Physics (purity > 99%, Lanzhou, China). Tween 80, Tween 60, oleic acid, Isopropyl myristate (IPM), propylene glycol and ethanol were purchased from Merck & Co Inc. All other chemicals and solvents were of analytical grade.

2.2. Animals

The Wistar rats (250–300 g) and Kunming mice (18–22 g) used in this study were supplied by the Experimental Animal Center of Kunming Medical University (License number: SCXK 2014-007), Kunming, China. The animals were housed at an ambient standard temperature, with a 12-h day/night cycle, and fed a standard pellet diet and water. The protocol of the experiments was carried out in accordance with the Code of Ethics of the World Medical Association and approved by the Experimental Animal Welfare and Ethics Committee of Kunming University of Science and technology.

2.3. Determination of the Den content

The determination of the Den was performed using methods which have been previously developed (Qiao et al., 2013). Briefly, the water stock solutions containing Dencichine standard (0.996 mg/mL) were prepared and diluted to appropriate concentrations for the construction of calibration curves and the validations of the analytical methods. After dilution and filtering was complete, 10 μL of the sample was injected into an Agilent Series 1200 (Agilent Technologies, USA) system, which was equipped with an Eprogen Synchropak WAX analytical column (4.6 × 250 mm; 6 μm; Darien, USA). The mobile phase contained 50 mM of NaH_2PO_4 aqueous solution (pH 4.0). The flow rate was 1.0 mL/min. The elution profile was monitored, and the peaks were identified by a UV detector at 213 nm and 30 °C. The calibration curves were constructed by plotting the peak areas *versus* the concentration of the drug. Meanwhile, validations of the methods were conducted, including linearity, precision, repeatability, and recovery.

2.4. Skin permeation experiment

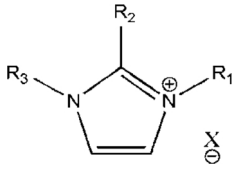
2.4.1. Preparation of the mice skin samples

The mice were euthanized by cervical dislocation. The subcutaneous fat and sub-dermal tissue were carefully removed from the abdominal skin with scissors, and cleaned with saline. The skin samples were then cut into appropriate sizes, and immediately used for the experiment. The animal experiment was reviewed and approved by Experimental Animal Welfare and Ethics Committee of Kunming University of Science and technology. All efforts were made to minimize animals' suffering, and to limit the number of animals used.

2.4.2. In-vitro skin permeation study

The drug aqueous solution (10 mg/mL) was prepared for the skin

Table 1
Chemical structures of the imidazolium ionic liquids used in the work.



$R_1 = C_2H_4COOH$	$R_2 = H$	$R_3 = -CH_3$	$X = Cl^-$	[HOOCEIM]Cl
$R_1 = C_2H_4OH$	$R_2 = H$	$R_3 = -CH_3$	$X = Cl^-$	[HOEIM]Cl
$R_1 = C_2H_4OH$	$R_2 = H$	$R_3 = -CH_3$	$X = PF_6$	[HOEIM]PF_6
$R_1 = CH_3$	$R_2 = H$	$R_3 = H$	$X = Cl^-$	[MIM]Cl
$R_1 = C_2H_5$	$R_2 = H$	$R_3 = H$	$X = Cl^-$	[EIM]Cl
$R_1 = CH_2(CH_2)_2CH_3$	$R_2 = H$	$R_3 = H$	$X = Cl^-$	[BIM]Cl
$R_1 = -CH_2(CH_2)_6CH_3$	$R_2 = H$	$R_3 = H$	$X = Cl^-$	[OIm]Cl
$R_1 = -CH_2(CH_2)_2CH_3$	$R_2 = H$	$R_3 = -CH_3$	$X = Cl^-$	[BMMIm]Cl
$R_1 = -C_2H_5$	$R_2 = H$	$R_3 = -CH_3$	$X = Cl^-$	[EMIm]Cl
$R_1 = -CH_2(CH_2)_2CH_3$	$R_2 = H$	$R_3 = -CH_3$	$X = Cl^-$	[BMIm]Cl
$R_1 = -CH_2(CH_2)_6CH_2$	$R_2 = H$	$R_3 = -CH_3$	$X = Cl^-$	[OMIm]Cl
$R_1 = -CH_2(CH_2)_2CH_3$	$R_2 = H$	$R_3 = -CH_3$	$X = PF_6$	[BMIm]PF_6
$R_1 = -CH_2(CH_2)_2CH_3$	$R_2 = H$	$R_3 = -CH_3$	$X = C_{12}SO_3$	[BMIM]$C_{12}SO_3$
$R_1 = -CH_2(CH_2)_2CH_3$	$R_2 = H$	$R_3 = -CH_3$	$X = H_2PO_4$	[BMIm]H_2PO_4