Original Article

การเปรียบเทียบสูตรตำรับอนุภาคสำหรับนำส่งเคอร์คูมินทางผิวหนัง: ลิโพโซม เฟร็กโซโซม และ อินเวโซม

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บทคัดย่อ

บทนำ: กว่าสี่สิบปีมาแล้วลิโพโซมถูกพัฒนาเพื่อเป็นตัวพานำส่งทางผิวหนังของยาหลากหลายชนิด อนุภาคมีหลายชนิด ได้แก่ ทรานสเฟอร์โซม เอทโธโซม เฟร็กโซมโซม อินเวโซม เมนโธโซม ทรานสอินเวโซม เป็นต้น โดยคุณสมบัติแท้จริงของอนุภาคขึ้นอยู่กับแต่ละส่วนประกอบ วัตถุประสงค์ของการศึกษานี้คือ เพื่อออกแบบและพัฒนาสูตรตำรับอนุภาคชนิดต่างๆ สำหรับการนำส่งเคอร์คูมินทางผิวหนัง **วิธีดำเนินการวิจัย**: เตรียมอนุภาคกักเก็บเคอร์คูมินที่มีประกอบคือ ฟอสโฟลิปิด คอเลสเตอรอล เคอร์คูมิน ในปริมาณคงที่ และแปรผันปริมาณของสารเพิ่มการซึมผ่านผิวหนัง (ทวีน 20 และเทอร์ปีน) โดยวิธีคลื่นเสียง ศึกษาคุณลักษณะทางเคมีกายภาพของสูตรตำรับอนุภาค (ได้แก่ ขนาดอนุภาค การกระจายขนาด ประจุ และประสิทธิภาพในการกักเก็บตัวยา) ศึกษาผลของสารเพิ่มการซึมผ่านผิวหนังต่อไขมันระหว่างเซลล์ของโครงสร้างขนาดเล็กด้วยเครื่องฟูเรียร์ทรานสฟอร์มอินฟราเรดสเปคโทรสโคปี (FTIR) และเครื่องดิฟเฟอเรนเชียลสแกนนิงแคลอริมิเตอร์ (DSC) **ผลการวิจัย**: ขนาดอนุภาคของทุกสูตรตำรับมีค่าน้อยกว่า 50 นาโนเมตร มีการกระจายขนาดแคบและมีประจุลบ ประสิทธิภาพในการกักเก็บตัวยาของเฟร็กโซมโซมและอินเวโซมมากกว่าลิโพโซมดั้งเดิมอย่างมีนัยสำคัญ แถบ FTIR และกราฟอุณหภูมิ DSC บ่งชี้ว่าสารเพิ่มการซึมผ่านผิวหนังอาจมีผลต่อคุณสมบัติการไหลของผิวหนัง **สรุปผลการวิจัย:** ผลการทดลองนี้บ่งชี้ว่า ทวีน 20 และเทอร์ปีนมีผลต่อคุณลักษณะทางเคมีกายภาพของสูตรตำรับอนุภาค เฟร็กโซมโซมและอินเวโซมคืออนุภาคที่เหมาะสมที่อาจใช้เป็นตัวพานำส่งทางผิวหนังของเคอร์คูมินได้

คำสำคัญ: เคอร์คูมิน, ลิโพโซม, เฟร็กโซโซม, อินเวโซม

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Comparison of Vesicle Formulations for Transdermal Delivery of Curcumin: Liposomes, Flexosomes and Invasomes

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Abstract

Introduction: For the past four decades, liposomes have been developed as transdermal delivery carriers in various drugs. There are several vesicle types, including transfersomes, ethosomes, flexosomes, invasomes, menthosomes and transinvasomes. The intrinsic properties of these vesicles depended on their compositions. The aim of this study was to design and develop the different vesicle formulations for the transdermal delivery of curcumin. **Methods:** Curcumin loaded vesicles containing a controlled amount of phospholipid, cholesterol, curcumin and various amounts of penetration enhancers (Tween® 20 and terpene) were prepared by using a sonication method. The physicochemical characteristics of vesicle formulations (e.g., vesicle size, size distribution, zeta potential and entrapment efficiency) were investigated. The effect of penetration enhancers on the intercellular lipid microstructure was also determined by the Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). **Results:** The vesicle size of all formulations was below 50 nm with narrow size distribution and negative zeta potential. The entrapment efficiency of flexosomes and invasomes was significantly higher than what is found in conventional liposomes. FTIR-spectra and DSC thermogram indicated that the penetration enhancers might result in the fluidity of the skin. **Conclusion:** These results indicated that Tween® 20 and terpene affected the physicochemical characteristics of the vesicle formulations. Flexosomes and invasomes are the optimal vesicle formulations that may be used as transdermal delivery carriers for curcumin.

Keywords: Curcumin, Liposomes, Flexosomes, Invasomes

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Introduction

Recent reports have remarked that curcumin (CUR) has diversed biological activities including anti-inflammatory, anti-oxidant and anti-cancer activity. Considering the biological activities via skin, CUR can be promoted for anti-aging, anti-inflammatory, psoriasis and wound healing (Chaudhary, 2013; Naksuriya, 2014). Unfortunately, the clinical use of CUR for topical application is limited due to its poor solubility in water and hence, minimal bioavailability via transdermal delivery. Novel vesicles with elastic characteristics have a special role in improving transdermal delivery of CUR. Previously, novel vesicle formulations, such as liposomes (Chen, 2012), transfersome (Agrawal, 2015) and ethosomes (Zhao, 2013) were prepared to improve the solubility, permeability, bioavailability and stability of CUR (Zhao, 2013).

However, the incorporation of surfactant (in transfersome) and ethanol (in ethosome) as potent penetration enhancers may have caused skin irritation (Lachenmeier, 2008; Wilhelm, 1994). The general requirements for the proper penetration enhancers should have been non-toxicity, non-irritating, non-pharmacological effect, and to provide reversible outcomes for skin. Terpenes, a group of potent penetration enhancers were found in essential oils that were extracted from natural products including flowers and fruits. Moreover, terpenes offered advantages over several penetration enhancers due to their natural origin as well as its Generally Regarded As Safe (GRAS) status. The aim of this study was to design and develop the different vesicle formulations for enhancing the transdermal delivery of curcumin using non-irritating penetration enhancers including nonionic surfactant (Tween® 20, T20) and a natural origin penetration enhancer (limonene; Lim). The physicochemical characteristics of vesicle formulations (e.g., vesicle size, size distribution, zeta potential and entrapment efficiency) were investigated. Moreover, the effect of penetration enhancers (T20 and Lim) on the intercellular lipid microstructure of skin model was determined by the Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC).

Materials and Methods

Materials

Curcumin (CUR), cholesterol (CHOL) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphatidylcholine (PC) was supplied as a special gift from LIPOID GmbH (Cologne, Germany). *d*-limonene (Lim) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Polysorbate-20 (Tween® 20, T20) was purchased from the NOF Corporation (Osaka, Japan). All other chemicals were commercially available and of analytical and high-performance liquid chromatography (HPLC) grade.

Vesicle preparation

CUR-loaded vesicle formulations (liposomes; CLP, flexosomes; FXS, invasomes; IVS) composed of a controlled amount of 10 mM phosphatidylcholine (PC), 1 mM cholesterol (CHOL), 5 mM CUR and various concentrations

of penetration enhancers (T20 and Lim) at 2% and 0.5-1.5%, respectively, were prepared. The different lipid composition of CUR-loaded vesicle formulations are shown in Table 1.

Table 1 Lipid compositions of different vesicle formulations

Form.	Composition (mM)				
	PC	Chol	CUR	T20	Lim
CLP	10	1	5	-	-
FXS	10	1	5	2%	-
IVS 0.5	10	1	5	2%	0.5%
IVS 1.0	10	1	5	2%	1.0%
IVS 1.5	10	1	5	2%	1.5%

CLP; conventional liposomes, FXS; flexosomes, IVS; invasomes, PC; phosphatidylcholine, Chol; cholesterol, CUR; curcumin, T20; Tween® 20, Lim; limonene

CUR-loaded vesicle formulations were prepared by using the sonication method (Duangjit, 2017). Briefly, the mixtures of PC, CHOL and CUR were dissolved in chloroform/methanol (2:1). The organic solvent was evaporated under a nitrogen gas stream. The freshly thin film was placed in a desiccator overnight to remove the remaining solvent. The dried thin film was hydrated with 0.1 M phosphate buffer solution (PBS, pH 7.4), and mixed with T20 and Lim. The vesicles were subsequently sonicated for two cycles of 15 min using a probe-type sonicator. The vesicle formulations were freshly prepared or stored in airtight containers at 4°C prior to use.

Physicochemical characterization

The vesicle sizes, size distributions and zeta potentials of the vesicle formulations were measured by photon correlation spectroscopy (PCS) (Zetasizer Nano series, Malvern Instruments, UK). Twenty microliters of the vesicle formulations were diluted with 1480 µL of deionized water. All measurements were performed in triplicate at 25°C.

Determination of CUR-loaded vesicles formulation

The concentration of CUR in the vesicle formulations was determined by HPLC analysis after disruption of the vesicles with Triton® X-100 (0.1% v/v) at a 1:1 volume ratio and was subsequently diluted appropriately with phosphate buffer saline, pH 7.4. The Triton® X-100/vesicle mixture was centrifuged at 10,000 rpm at 25°C for 10 min. The supernatant was filtered with a 0.45 µm nylon syringe filter. The entrapment efficiencies (EE) of the CUR-loaded vesicle formulation were calculated according to the following equation:

$$\% \text{ EE} = (C_L/C_i) \times 100 \qquad (1)$$

where C_L is the concentration of the CUR-loaded vesicle formulation, as described in the above methods, and C_i is the initial concentration of CUR added to the formulation.

HPLC analysis

The concentration of CUR in all samples was analyzed using a HPLC (ThermoDionex UV/Vis, USA). The analytical column was Hyper

Clone 5u ODS C-18 column (250x4.6 mm), and the mobile phase consisted of acetronitrile/0.1% phosphoric acid (65 : 35) under the isocretic mode at 40°C, was set at a flow rate of 1.5 mL/min, while the UV detector was set at 425 nm for all determinations. The calibration curve for CUR was in the range of 0.01-100 μg/mL with a correlation coefficient of 0.999. The data was reported as mean ± S.D. (n=3).

Skin characterization

Following the *in vitro* skin permeation study for 24 h, the skin model of shed snake skin was washed with water and blotted dry. The spectrum of the skin model was recorded in the range of 500-4000 cm^{-1} using a Fourier transform infrared (FTIR) spectrophotometer (Nicolet 4700, Thermo Scientific, USA). The same shed snake skin was weighed (2 mg) and cut into small pieces, and put to an aluminum crimp pan. The shed snake skin was used as a skin model to investigate the possible mechanisms. The effect of the penetration enhancers on the microstructure of the skin model was confirmed by the differential scanning calorimetry (DSC) (Pyris Sapphire DSC, PerkinElmer instrument, USA). The samples were heated from 25 to 350 ˚C at a heating rate of 10˚ C/min. All DSC measurements were collected under a nitrogen atmosphere with a flow rate of 30 mL/min.

Data analysis

The data was reported as the means ± standard deviation (SD) (n=3), and statistical analysis of the results was carried out using one-way ANOVA followed by an LSD post hoc test. A *p*-value of less than 0.05 was considered to be significant.

Results

The physicochemical characteristics of different CUR-loaded vesicle formulation are shown in Figure 1. The results indicated that the vesicle composition significantly influenced the physicochemical characteristics of CUR-loaded vesicle formulations. A previous study reported that the stability of the vesicle's bilayer increased when Chol was incorporated. The bilayer permeability of the vesicles could be controlled by inducing conformational organizing of the hydrocarbon chains of the bilayer core (Raffy and Teissié, 1999). Hence, all CUR-loaded vesicle formulations in this study were composed of Chol.

The vesicle size of all CUR-loaded vesicle formulations was smaller than 50 nm with a narrow size dispersion (less than 0.4) (Figure 1A). The small vesicle size of liposomes (less than 120 nm) was an appropriate size for transdermal drug delivery carriers as compared to the large ones (Verma, 2003). These results migh confirm that the lipid composition and the method of preparation in this study were prospered condition for preparation the nano-size CUR-loaded vesicle formulations with narrow size dispersion. The incorporation of T20 significantly decreased the vesicle size of FXS compared to CLP. On the other hand, the incorporation of Lim led to an increase in the vesicle size of IVS. A previous study reported that the incorporation of terpenes (limonene, cineole, geraniole) did not affect the vesicle size (Subongkot, 2012).

However, the vesicle size was reported to increase when the concentration of terpenes

(Lim) in the vesicle formulations increased. Vesicles containing 0.5% of Lim were 93 nm and 1% Lim were 124 nm (Dragicevic-Curic, 2008).

Recently, it has been reported that the vesicle size of the finasterode-loaded invasome was affected by molecular size of terpene, wherein invasomes with carvone (molecular size 150.22 g/mol) were about 4,540 to 4,800 nm and invasomes with nerolidol (molecular size 222.37 g/mol) were about 11,230 to 13,000 nm in size (Prasanthi and Lakshmi, 2013). The vesicle sizes of nimesulide-loaded liposome with different terpenes were 164 nm (control), 194 nm (with citral), 216 nm (with limonene) and 244 nm (with cineole) (Badran, 2012). Therefore, vesicle size might be influenced by the molecular size of the terpene incorporated (Prasanthi and Lakshmi, 2013) and the concentration of terpene added (Dragicevic-Curic, 2008). The response surface of the capsaicin-loaded transinvasomes indicated that with the addition of Lim up to 1.5%, the vesicle size may increase to more than 180 nm. Therefore, to formulate the vesicle formulation under 100 nm, the 1.0% Lim or lower should be conducted (Duangjit, 2017). However, the contradictory effects of terpenes on the vesicle size, and the relationship of the intrinsic properties of the model drug should be investigated in further studies.

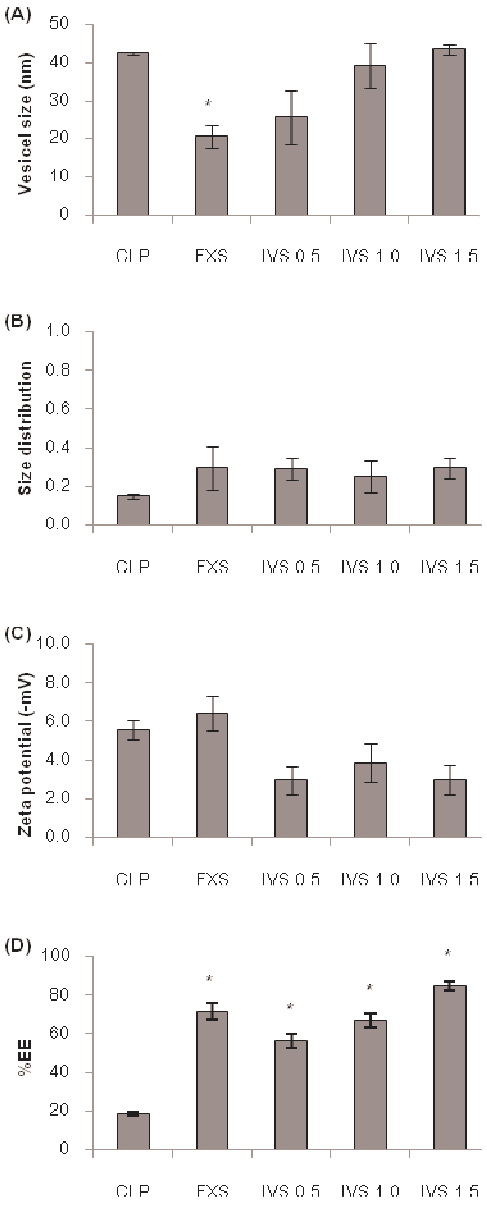

Figure 1 The physicochemical characteristics of different CUR-loaded vesicle formulations: (A) vesicle size, (B) size distribution, (C) zeta potential and (D) entrapment efficiency. Each value represents the mean±S.D. (n=3), * $p<0.05$ compare to CLP.

The size distribution of all CUR-loaded vesicle formulations was less than 0.4 (Figure 1B); thus, the lipid composition and the method of preparation were appropriate for further research.

The zeta potential of all CUR-loaded vesicle formulations was negative (-3 to -7 mV) as shown in Figure 1C, depending on the intrinsic properties of vesicle compositions and total net charge. The CUR had three pKa: approximate pKa_1 at 8.5, pKa_2 at 9.8 and pKa_3 at 10.5 (Bernabe-Pineda, 2004; Hatcher, 2008), The CUR had no charge at the experimental condition. Since the isoelectric point (PI) of PC as a zwitterionic compound at the experimental pH 7.4 was 6, it should be presented as an anionic species in this study. Therefore, the total net charge of all vesicle formulations might be influence the PC. A previous study suggested that negatively charged vesicles can potentially enhance the skin penetration of betamethasone and betamethasone dipropionate (Gillet, 2011). Moreover, the skin permeation rate of the melatonin- and betahistine-loaded negatively charged liposomes was significantly higher than that of positively charged liposomes. The skin permeation of ethosuximide-encapsulated negatively charged liposomes were also slightly higher than that from positively charged liposomes (Ogiso, 2001). Thus, CUR-loaded vesicles with negative charge may be desirable for skin penetration.

The entrapment efficiency (EE) of the CUR-loaded vesicle formulations were dependent on their vesicle compositions. The EE of FXS and IVS was significantly higher than that of CLP. In a previous study, Duangjit et. al. (2014) noted that the drug content, entrapment efficiency and penetration enhancer's concentration were major factors important to the skin permeablity. The increase in the drug content and the penetration enhancer's concentration to high values resulted in high skin permeability of meloxicam-loaded liposomes (Duangjit, 2014). Therefore, the high skin permeability of CUR-loaded vesicle formulation could be approached by the high EE of FXS and IVS formulations.

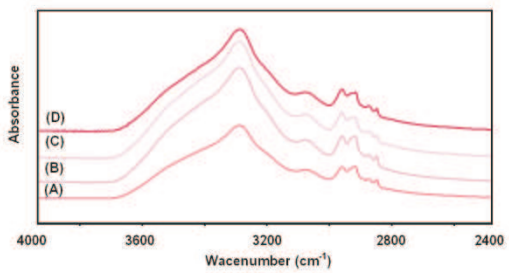

Figure 2 The FTIR spectra of the skin model treated with (A) PBS, (B), 50% ethanolic solution (C) 2% T20 and (D) 2% Lim.

The skin treated with PBS, 50% ethanolic solution, 2% Tween® and 2% Lim was characterized using FTIR and DSC. The change in the microstructures of the intercellular lipids occurred following the treatment of skin with the penetration enhancers, as shown in the FTIR spectra and DSC thermograms. The FTIR peaks from the absorption broadened C–H (CH_2) asymmetric and symmetric stretches were near 2,919.258 cm^{-1} and 2,848.546 cm^{-1}, respectively. The peaks of the FTIR spectra of the skin treated with various penetration enhancers shifted to 2,919.257 cm^{-1} (PBS), 2,925.146 cm^{-1} (50% ethanololic solution) 2,919.256 (2% T20), 2,919.255 cm^{-1} (2% Lim) and 2,851.546 cm^{-1} (50% ethanololic solution).

Meanwhile, the DSC thermograms also displayed peak shifts, from 225.48°C for the intact skin sample treated with PBS (as the control) to a lower transition temperature for the skin sample treated with various penetration enhancers. The intercellular lipid of the skin sample existed in the liquid state (Duangjit, 2014). The shifted peak of the skin samples was 225.58 °C (50% ethanololic solution), 223.64 °C (2% T20) and 227.34 (2% Lim), depending on the penetration enhancers.

The present study revealed that the intercellular lipid arrangement of the skin sample treated with various penetration enhancers was disrupted by altering the fluidity of the intercellular lipids. Thus, the interruption of the intercellular lipids by these penetration enhancers or by the vesicle components may have caused an increase in the skin permeability of CUR. However, the skin permeation profile of the skin treated with different CUR-loaded vesicle formulations should be used to confirm the skin permeability of each vesicle in further studies.

Discussions and Conclusion

These results indicate that T20 and Lim affected the physicochemical characteristics of the vesicle formulations. The physicochemical characteristics of the vesicle formulations (both flexosomes and invasomes) such as vesicle size, size distribution, zeta potential and entrapment efficiency may be important factors that should be considered intensively in the development of a vesicle formulation for transdermal drug delivery due to characteristics being the primary factors that significantly correlated with the efficacy of the optimal vesicle formulation.

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