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# Ethosomes as delivery system for transdermal administration of vinpocetine

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The purpose of the present study was to develop a novel transdermal vinpocetine patch containing a stable formulation and with good entrapment efficiency, and percutaneous absorption which via ethosome. Ethosome was found to be a more efficient delivery carrier with high encapsulation capacities (79.5% ± 1.8%) and nanometric size (180.7 ± 1.5 nm). *In vitro* percutaneous permeation experiments demonstrated that the permeation of vinpocetine through abdominal skin of Sprague Dawley was significantly increased when ethosome was used. The vinpocetine transdermal fluxes from ethosome gel (3.56 ± 0.13 μg/cm$^2$/h) were 6.72 and 3.10 times higher than that of vinpocetine gel solution and vinpocetine aueous solution, respectively. Furthermore, the $AUC_{0\rightarrow\infty}$, and eliminiation half-life by the transdermal administration were significantly higher than those by the intragastric administration ($P < 0.01$). The study demonstrated that ethosome is a promising vesicular carrier for enhancing percutaneous absorption of vinpocetine.

Vinpocetine (Vin) is an alkaloid extracted from Apocynaceae plants, and is also a derivative of vincamine. It is widely used for the prevention and treatment of various cerebrovascular diseases and ischaemic stoke (Bereczki and Fekete 1999; Feigin et al. 2001). However, the oral administration of Vin is limited by its poor absorption and a remarkable first pass effect. The patient compliance is also greatly reduced because of the poor oral bioavailability (~7%) and the short $t_{1/2}$ leading to frequent drug dosing (El-Laithy et al. 2011). Furthermore, Vin is a poorly water-soluble drug (5 μg/ml). To overcome these problems, it is necessary to find a proper route of administration and to prepare an effective delivery system of Vin. Transdermal administration could avoid the extensive first-pass effect that metabolizes 75% of orally administered Vin (Hua et al. 2004). Touitou et al. (2000) reported that ethosomes could enhance drug transport through the skin. Due to their special structural properties, ethosomes possess high entrapment efficiency (Bhalaria et al. 2009), and can be used to improve the solubility of poorly water soluble drugs and enhance their permeation through the skin (Dubey et al. 2007). In addition, they could reduce the overall dose and decrease toxicity while maintaining the effectiveness of the drug (Bendas and Tadros 2007). In the present work, we concern the preparation and pharmacokinetics of ethosomes as a novel carrier for the transdermal delivery of Vin.

**Table: Permeation parameters of vinpocetine through Sprague Dawley rat skin (n = 3)**

| Formulations | $J_{ss}$ | $P_{app} \times 10^3$ | ER |
|---|---|---|---|
| Aqueous solution | 1.15 ± 0.14 | 26.38 ± 3.22 | 1 |
| Gel solution | 0.53 ± 0.03 | 12.16 ± 0.81 | 0.46 |
| Ethosome gel | 3.56 ± 0.13 | 81.65 ± 2.98 | 3.10 |

$J_{ss}$: steady state flux (μg/cm$^2$/h); $P_{app}$: apparent permeability coefficients (cm/h); ER: enhancement ratio.

The particle size, entrapment efficiency and loading content of Vin ethosomes were 180.7 ± 1.5 nm (polydispersity index: 0.127 ± 0.08), 79.5% ± 1.8% and 3.52 mg/ml respectively and there were no significant changes when Vin ethosomes were sealed in 10 ml clear glass vials and stored at room temperature (25 ± 1 °C) for four weeks. The results suggested that ethosomes might favor transdermal delivery of Vin.

The penetration profiles of the three formulations of Vin are shown in the (Fig. A). The amount of Vin permeation through the abdominal skin of Sprague Dawley rats *in vitro* from the ethosome gel was significantly ($P < 0.01$) higher than that from the other two formulations. The Vin transdermal fluxes from ethosome gel (3.56 ± 0.13 μg/cm$^2$/h) were 6.72 and 3.10 times higher than that of Vin from gel solution and aqueous solution (Table), which suggested that ethosomes could enhance permeation of the drug through skin. Due to the hydrophobicity and low solubility of Vin, the permeabilities of Vin from gel solution and aqueous solution were very low.

The Vin plasma concentration-time curves following the administration of Vin ethosome gel and intragastric administration of Vin oral tablet in rats are shown in the Fig. (B). Remarkable differences in the shape of the plasma concentration-time courses between the two treatments were found, expressed by rapid sharp peak of Vin absorption from the oral tablet at 1.0 h followed by a fast decline of Vin plasma levels. Compared with the oral tablet, the absorption of Vin from the ethosome gel was much slower and extended over a longer period of time where the large fraction of its drug content was delivered during the first 10 h of the application period, followed by a slower release phase that extended the presence of Vin in plasma for about 24 h. Moreover, the ethosome gel led to higher Vin plasma levels from 4 h to 12 h compared to the oral tablet. Vin plasma concentration–time data following the two treatments in rats were fitted by the non-compartmental analysis using WinNonlin software (vision 5.2) according to the report of El-Laithy et al. (2011). The $C_{max}$, $AUC_{0\rightarrow\infty}$ and $t_{1/2}$ were 0.47 ± 0.02 μg/ml, 7.27 ± 0.47 μg·ml$^{-1}$·h and 5.56 ± 0.78 h in group A, 0.97 ± 0.06 μg/ml, 3.29 ± 0.10 μg·ml$^{-1}$·h and 2.19 ± 0.18 h in group B, respectively. The relative bioavailability of Vin ethosome gel to the oral tablet was on average 220.9%. The results suggested that transdermal administration of Vin ethosome could maintain stable and sustained plasma drug concentrations, increase bioavailability, reduce application times and strengthen treatment efficacy. Therefore, ethosome gel is a promising vesicular carrier for enhancing percutaneous absorption and treatment efficacy of Vin.

## Experimental

The preparation of ethosome gel was based on the report of Zhaowu et al (2009), and was slightly modified. Ethosome gel was composed of 3% (w/w) lecithin, 0.2% (w/v) cholesterol, 45% (w/w) ethanol, vitamin E (0.06% w/w), Vin and water. Cholesterol, Vin, vitamin E and lecithin were dissolved in

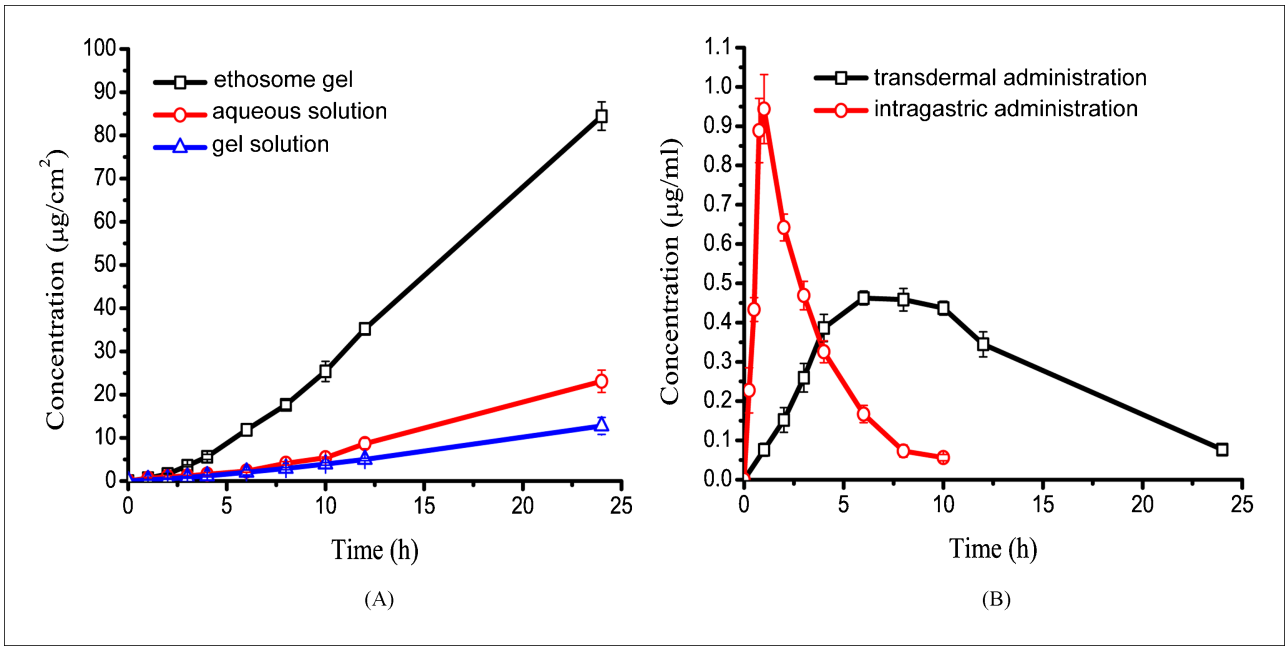


Fig.: Penetration profile (A) of vinpocetine permeated through Sprague Dawley rat skin from different formulations (mean ± SD, n = 3) and plasma concentration-time profile (B) following administration of 5 mg vinpocetine from ethosome gel and vinpocetine tablets (mean ± SD, n = 6)

ethanol, and then the coarse dispersion was produced by adding slowly the water into the ethanol solution under constant stirring. To further reduce the particle size, the coarse dispersion was homogenized with an ultrasonic probe (JY92-II, Ningbo Scientz Biotechnology CO., LTD, China) for 20 times (working time: 3 s, intermittent time: 3 s, work power: 200 W). The particle size of the ethosome dispersion was measured by photon correlation spectroscopy using a Malvern Zetasizer (Nano-ZS90, Malvern Instruments, UK). To prepare Vin ethosome gel, Carbopol 934P (1% w/w) was dispersed in the deionized water and stored for 12 h to swell sufficient, and then pH value was adjusted to 7 using triethanolamine solution (Esposito et al. 2005). Vin ethosome was diluted with an appropriate amount of carbopol gel to obtain Vin ethosome gel.

Excised abdominal skin of Sprague Dawley rats was mounted on a Franz diffusion cell (effective permeation area was 0.785 $cm^2$, receptor compartment volume was 2 ml). The receptor compartment contained an ethanol-water solution (30:70 v/v) to allow the establishment of the "sink condition". The solution was stirred (200 r $min^{-1}$) with a magnetic stirrer at 37 ± 0.3 °C during all the experiments (Liu et al. 2011). One point seven grams of Vin ethosome gels were placed on the skin surface and the donor compartment was sealed to avoid evaporation. Samples (0.5 ml) were withdrawn at predetermined time intervals (t = 0, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h) from the receptor compartment and the cell was refilled with an equivalent amount of fresh receptor solution. Samples were filtered through a 0.45 μm microporous membrane and then were analyzed using HPLC.

Vin fluxes through the skin were calculated by plotting the cumulative amount of drug penetrating the skin against time. $J_{ss}$ (μg/$cm^2$/h) was calculated according to the formula, $J_{ss}$ = dM/dt. Where M is the cumulative amount of Vin permeated through skin per unit area (μg/$cm^2$) at experimental time (Higashiyama et al. 2004; Williams and Barry 2012). Enhancement ratio was calculated according to the expression, $ER = J_{eth}/J_{sol}$. Where $J_{eth}$ is the flux of Vin ethosome gel and $J_{sol}$ is the flux of Vin gel solution or aqueous solution (Jia-You Fang 2001).

*In vivo* studies were carried out to compare the pharmacokinetic characteristics of ethosome gel containing 5 mg Vin (group A) to an oral commercial tablet containing the same dose of Vin (group B). Twelve healthy male Sprague Dawley rats were randomly divided into group A and B. For group A, 0.5 ml blood (with heparin) was collected from intraocular angular vein at predetermined time intervals (t = 0, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h). For group B, the difference from group A was the predetermined time intervals (t = 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, and 10 h). The blood was centrifuged for 15 min at 5000 r $min^{-1}$ to withdraw plasma. Fifty microlitres NaOH solution (0.5 M) was added into 200 μl plasma and the mixture was swirled for 2 min. After that, 2 ml ether was added to the mixture and swirled for 2 min. After centrifugation at 4000 r $min^{-1}$ for 5 min, the supernatant was withdrawn to another centrifuge tube. Repeat again, and merge two extracting solutions before the extracting solution was evaporated under nitrogen at 37 °C. Finally, fifty microlitres of methanol were added to dissolve the residues and swirled for 3 min, and then 20 μl of the resulting solution was injected into the HPLC for analysis.

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