Penetration effect of Ostrich Oil as a Promising Vehicle on Transdermal Delivery of Sinomenine

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Abstract: The present study investigated the feasibility of ostrich oil utilizing as a promising vehicle for improved skin permeation of sinomenine with reference to vaseline matrix containing different content of chemical enhancers. The fatty acid composition of ostrich oil was analyzed by GC-MS. Penetration enhancing potential of ostrich oil on permeation of sinomenine across rat abdominal skin *in vitro* was studied using an automatic diffusion cell apparatus. The content of sinomenine percutaneous absorbed was determined by HPLC. Various parameters viz. steady-state skin flux (J_{ss}), permeability coefficient (k_p), cumulative amount of sinomenine (Q) and enhancement ratios (ER) were calculated from the permeation data. Fick's law of diffusion and Scheuplein kinetic were used to evaluate the transdermal absorbent enhancement of ostrich oil to sinomenine. Ostrich oil showed significant penetration effect on sinomenine compared with vaseline matrix containing different content of chemical enhancers, the density sequence as follow: 2% Azone > ostrich oil > 1% Azone plus 1% propylene glycol > 1% Azone > 3% Azone > 1% propylene glycol. The percutaneous endosmic rate constant (J_{ss}) and permeability coefficient (k_p) of sinomenine in ostrich oil through rat skin were 10.01 $\mu\text{g}/\text{cm}^2/\text{h}$ and 0.087, respectively. Ostrich oil produced stronger enhancement ($\text{ER} = 24.31$) with greater cumulative amount of drug permeated (255.53 $\mu\text{g}/\text{cm}^2$) up to 24 h and caused no skin irritation. The drug release of sinomenine was coincided with Fick's equation. In summary, ostrich oil containing fatty acids is proposed as a promising adjuvant for use in cosmetics and pharmaceuticals for improved permeation of drug.

Key words: ostrich oil, sinomenine, transdermal, penetration vehicle, fatty acid

1 INTRODUCTION

Sinomenine (Fig. 1) is a bioactive alkaloid derived from the Chinese medicinal plant, *Sinomenium acutum*, which has been widely used to treat inflammatory and arthritic diseases for one thousand years. However, oral administration of sinomenine is often limited on account of gastrointestinal adverse side effects, including nausea, diarrhea, constipation, gastralgia, and occasionally vomiting. In addition, the oral route of drug delivery is not preferable for patients with low oral bioavailability; also, the short elimination half-life of sinomenine requires frequent dosing¹⁾. Hence, the transdermal route could be a better alternative route, because it bypasses first-pass metabolism, minimizes the gastrointestinal side effects, increases patient compliance, maintains a constant drug level in plasma and physiological effects.

An essential prerequisite for transdermal absorption of drugs is that the drug must be capable of passing through the skin at a sufficiently high rate to achieve therapeutic

Fig. 1 The chemical structures of sinomenine.

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plasma concentrations. However, the major barrier to the percutaneous transport of drugs across the skin is stratum corneum (SC)²⁾. One of the most widely used strategies to deliver an effective dose of drug through skin is to reversibly reduce the barrier function of the skin with the aid of penetration enhancers³⁾. Fatty acids have been shown to interact with the stratum corneum lipids, and a number of fatty acids have been identified as skin permeation enhancers^{4, 5)}. Ostrich oil, a natural bird fat oil containing fatty acids, has been used for alleviating various dull pains caused by arthritis and cervical spondylosis since ancient times, exhibiting the remarkable curative effect on aches and diminishing inflammation⁶⁾. Meanwhile, it has been used as an ideal adjuvant in cosmetics and pharmaceuticals owing to its remarkable permeability, excellent lubrication and emulsion properties⁷⁾.

In this study, ostrich oil was investigated as the test vehicle as it is an inexpensive and rich source of natural fat oil. Sinomenine was selected as the model drug, and rat abdominal skin was removed hairs and used *in vitro* penetration experiment. Various contents of Azone, propylene glycol (PG) and the combination of Azone and PG have been used as reference enhancers in vaseline matrix. The fatty acid composition of ostrich oil was analyzed by GC-MS. The influence and mechanism of ostrich oil on the transdermal delivery of sinomenine were also evaluated.

2 EXPERIMENTAL

2.1 Materials

Standard of sinomenine (purity > 99%) was purchased from the Chinese Authenticating Institute of Material and Biological Products (Beijing, China). Acutum alkaloid extracts that contain 98% sinomenine (w/w) was kindly provided by Zelang Medical Technology Co., Ltd (Nanjing, China). Ostrich oil was extracted from ostrich fat by supercritical fluids extraction (SFE) based on previously described procedures⁴⁾. Azone, propylene glycol, stearic acid, triethanolamine and lanolin were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Methanol of HPLC grade was obtained from Amethyst Chemicals J & K Scientific Ltd. All other reagents used were of analytical grade. Deionized water purified by a Milli-Q water-purification system was used in all experiments.

2.2 Animals

Male Sprague-Dawley rats (8 weeks old, 200-220 g) were supplied by Central Animal House Facility of Zhejiang University and kept under standard laboratory conditions in 12 hr light/dark cycle at temperature $25 \pm 2^\circ\text{C}$ and humidity 70-75%. Animals were provided with standard rodent chow and water ad libitum.

2.3 Analytical Methodology

Samples in solubility and permeation studies were analyzed by Agilent 1200 series HPLC system (Agilent Technologies, USA) with PAD detector. HPLC analysis was performed according to the method of China Pharmacopoeia 2010 edition part I. The mobile phase was methanol: 0.005 M phosphate buffered (55:45, v/v) adjusted to pH 9.0 with triethylamine. Volume of sample injected was 20 μL . Mobile phase was run at a flow rate 1 ml/min through a reverse phase C-18 column (250 mm $\times$ 4.6 mm, 5 μm , Agilent Technologies, USA). Detection wavelength was set at 265 nm, and the retention time of sinomenine was 6.439 min. The calibration curve was made by standard of sinomenine for sample analysis.

For the identification of fatty acids composition in ostrich oil, the GC method described in a previous study was used⁴⁾. The GC-MS system consists of an Agilent 6890N GC equipped with a split-splitless injector coupled with an Agilent 5975I MS equipped with an EI ion source and a quadrupole array detector. In the GC assay, the column oven temperature was programmed from 130 to 170 $^\circ\text{C}$ at 5 $^\circ\text{C}/\text{min}$, holding for 30 min, and then heated to 270 $^\circ\text{C}$ at 30 $^\circ\text{C}/\text{min}$, holding for 7 min. The temperatures of quadrupole, ion source chamber, injector and additional channel were 150, 230, 250 and 280 $^\circ\text{C}$, respectively. The carrier gas, helium (99.99%) was adjusted to a linear velocity of 1.0 mL/min. The ionization energy was 70 eV with the total scan mode and the MS scan interval was 40-500 amu.

2.4 Solubility Studies

Solubility study was performed according to the method of Rahul Jain *et al.*⁸⁾. Briefly, an excess amount of sinomenine was added to water and PG and shaken at $37 \pm 0.5^\circ\text{C}$ for more than 48 hours until equilibration. The suspensions were centrifuged at 8,000 rpm for 15 min. The supernatant was then filtered through 0.45 μm membrane filters and the concentration of drug was measured by HPLC after appropriate dilution. The experiments were performed in quadruplicate and the results were shown in Table 1.

2.5 Partition Coefficient determination

n-Octanol and water were mutually saturated for 24 hours before the experiment. Octanol/water partition coefficient ($P_{o/w}$) of sinomenine was determined by shake flask method that was based on thorough mixing of the two phases to reach the equilibrium^{9, 10)}. Stock solutions of sinomenine were made in aqueous solution with pH of 2, 3, 4, 5, 6, 7, 8, 9, 10 adjusted with hydrochloride solution or sodium hydroxide solution, and then presaturated with the analytic grade *n*-octanol. Ten milliliters of stock solutions were merged with 10 ml of *n*-octanol phase presaturated with water, and the phases of the solvent system were mutually saturated by shaking for 48 h on a mechanical shaker at the temperature of 25 ± 0.5 . After centrifugation at 6,000

rpm for 15 min, sinomenine concentrations in each phase were determined by HPLC method. Three independent measurements were conducted with all solutions. Apparent partition coefficient P was calculated according to the literature¹¹⁾.

2.6 Preparation of formulations

The compositions of blank control formulation were vaseline as vehicle and sinomenine (5 mg/g) without either penetration enhancers and ostrich oil. Control formulations were prepared utilizing vaseline as the matrix with different penetration enhancers such as 1% Azone, 2% Azone, 3% Azone, 1% Azone + 1% PG and 1% PG, respectively. Sinomenine (5 mg/g) was then added to control formulations. Test formulation was prepared by the same method except that ostrich oil was used as vehicle without penetration enhancers.

2.7 *In vitro* Skin Permeation studies

2.7.1 Preparation of Isolated Rat Abdominal Skin

The rats were sacrificed by giving excess ether anesthesia. The hairs from the abdominal surface of the rat were removed by a clipper and isolated skin was surgically removed from the rats¹²⁾. The subcutaneous tissue adhering to the skin was separated with help of scalpels and the dermis side was wiped with isopropyl alcohol to remove the residual adhering fat. The skin was washed with normal saline and stored in a deep freezer at -20°C until use. The skin was examined under light microscope before the permeation experiments to ensure the integrity of the skin^{13,14)}.

2.7.2 Diffusion of sinomenine across rat skin in automatic diffusion apparatus

The permeability of sinomenine across rat skin *in vitro* was studied using automatic diffusion apparatus (Pharmacopoeia Standard Instrument Factory of Tianjin, China) fitted with modified Franz diffusion cells at $37 \pm 0.5^{\circ}\text{C}$ through the use of a circulating water bath. The diffusion cell consisted of donor and receiver chambers each of capacity 7 mL with a diffusional area of 1.65 cm^2 . The treated skin pieces were mounted over diffusion cells with the dermal side in contact with the receptor phase. The receptor compartments were filled with phosphate buffered saline (PBS, pH 6.8) containing 0.02% w/v of sodium azide to retard microbial growth. The receptor phase was stirred at 500 rpm with a small magnetic bar to mix the concentrations uniformly. Skins were allowed to equilibrate for 1 h before experimentation.

The stratum corneum faced the donor chamber filled with 2.0 g test cream containing sinomenine (5 mg/g). Samples (2 mL) were withdrawn from the receiver solution at predetermined time intervals (1, 2, 4, 6, 8, 10 and 24 h). Sinomenine concentrations were determined by the HPLC method. Sample volume was immediately replaced to their marked volumes with fresh receptor medium (maintained

at $37 \pm 0.5^{\circ}\text{C}$) after each sampling. Addition of solution to the receiver compartment was performed with great care to avoid trapping air beneath the dermis samples. All experiments were performed in quadruplicate.

2.8 Data Analysis

The cumulative amount of drug (Q , $\mu\text{g}/\text{cm}^2$) permeated through a unit area was plotted as a function of time. The steady state flux (J_{ss} , $\mu\text{g}/\text{cm}^2/\text{h}$) was determined as the slope obtained from the linear regression portion of the plot. The lag time (T , h) was determined by extrapolating the linear portion of the curve to the abscissa. Permeability coefficient (K_p , cm/h) was calculated from the ratio of flux to drug concentration in the donor chamber. In addition, permeation profiles were also analyzed by the skin concentration of drugs ($\mu\text{g}/\text{g}$). The ER was calculated from the K_p and skin concentration of drugs with enhancer divided by the same parameter without enhancer (control). All percutaneous permeation data are mean $\pm$ S.D. Statistical significance was checked by student's t test and considered to be granted at $p < 0.05$.

3 RESULTS AND DISCUSSION

3.1 Physicochemical Properties

The physicochemical properties of the permeants, the molecular weight, solubility and $\log P_{o/w}$ of drugs, are thought to play an important role in determining the promoting activity of penetration enhancers on the permeation of the drug across the skin^{3,15)}. In this study, the molecular weight of sinomenine is 329.38 g/mol (Table 1), which is in the relatively range of 200-500 g/mol. The drug in this molecular weight range has a potential in transdermal delivery¹⁶⁾. The solubility of the drug, which depends on the chemical structure of itself and the vehicle, is important in determining the rate of delivery into the skin. As can be seen from Table 1, sinomenine has higher solubility in water at $87.24 \pm 8.93\text{ mg/mL}$ and moderately solubility in PG at $61.36 \pm 5.16\text{ mg/mL}$, respectively. Contrarily, it possesses low solubility in *n*-octanol at $3.71 \pm 0.24\text{ mg/mL}$.

The partition coefficient, $P_{o/w}$, was defined as the ratio of

Table 1 Physicochemical Properties of sinomenine.

Properties	Results
Molecular weight (g/mol)	329.38
Water solubility (mg/mL)	87.24 ± 8.93
1, 2-PG solubility (mg/mL)	61.36 ± 5.16
PBS (mg/mL)	114.97 ± 9.61
Partition coefficient ($P_{o/w}$)	0.043
Solubility parameter ($\text{MPa}^{1/2}$)	24.42

the concentration of the compound in two immiscible liquid phases such as *n*-octanol and water. $P_{o/w}$ obtained from above calculation method, was 0.043. For practical purposes, the logarithm of the partition coefficient ($\log P_{o/w}$) which is a measure of how well a substance partitions between a lipid and water, determines the route of drug penetration through the skin. By comparison, $\log P_{o/w}$ (-1.37) was not in the specified scope ($2\sim3$). The possible reason was that sinomenine molecule had considerable water solubility (87.24 ± 8.93 mg/mL, Table 1).

In summary, sinomenine had a low potential of permeating through the skin to the target, therefore, some additives such as permeation enhancer should be used in order to increase the permeation ability of drug.

3.2 Fatty acid composition of ostrich oil

Detailed identification and quantization of the fatty acid composition in the ostrich oil produced by SFE were performed by GC-MS. As shown in Fig. 2 and Table 2, 18 compounds were detected in the ostrich fat, among which 15 compounds were identified as fatty acids ($99.90 \pm 0.92\%$). The fatty acid composition of ostrich fat was characterized by 9-octadecenoic acid and hexadecanoic acid as the most major fatty acids. 9-octadecenoic acid ($40.7 \pm 0.3\%$) was the principal unsaturated fatty acid, followed by 9, 12-octadecadiene acid ($7.38 \pm 0.02\%$) and 9-hexadecenoic acid ($7.13 \pm 0.15\%$). Hexadecanoic acid ($32.5 \pm 0.3\%$) was the predominant saturated fatty acid, followed by octadecanoic acid ($7.43 \pm 0.05\%$). These five fatty acids contributed to about 95.12%, and other ten minor fatty acids only made up 4.78% approximately. Many fatty acids are generally

recognized as safe and are approved by the FDA as inactive ingredients in cosmetic products. The effects of fatty acids as permeation enhancers have been shown to be dependent on their structure, alkyl chain length, and degree of saturation¹⁷. Unsaturated fatty acids have been shown to promote higher magnitudes of permeation enhancement across skin when compared to saturated fatty acids of the same chain length. This has been attributed to the higher disrupting nature of the kinked chain of these fatty acids that would result in a higher magnitude of lipid disruption^{18–20}.

3.3 *In vitro* Percutaneous Permeation of sinomenine

To further evaluate the penetration effect of ostrich oil as a vehicle *in vitro*, the classic permeation enhancers such as Azone and propylene glycol (PG) were chosen as positive reference-enhancing promoter in the control formulations. Previous studies demonstrated that Azone is generally used at low concentrations (1–5% w/v), which can increase percutaneous permeation of various drugs²¹. PG has been extensively used as a solvent for transdermal formulation, which acts as a solvent for various compounds leading to an increased thermodynamic activity and enhanced “solvent drag” of the drug molecule.

Fig. 3(A) and (B) are examples of HPLC chromatograms resulting from analysis of the effect of ostrich oil on permeation of sinomenine across rat skin after 1 h and 24 h. A significant increasing height of the peak in sample collected from 1 to 24 h indicated that ostrich oil has higher permeation enhancement on sinomenine. Permeation profiles for sinomenine across rat abdominal skin in ostrich oil and control formulations containing other permeation enhanc-

Fig. 2 GC chromatograms of ostrich oil obtained by SFE.

Table 2 The fatty acids composition of ostrich oil.

No.	Fatty Acid	Molecular Formula	Relative amount (%)
1	Unidentified		0.03 ± 0.00
2	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	0.02 ± 0.00
3	Unidentified		0.02 ± 0.00
4	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	0.65 ± 0.02
5	Pentadecanoic acid	C ₁₅ H ₃₀ O ₂	0.10 ± 0.00
6	7-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	0.21 ± 0.01
7	9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	7.13 ± 0.15
8	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	32.5 ± 0.3
9	Heptadecanoic acid	C ₁₇ H ₃₄ O ₂	0.11 ± 0.00
10	9,12,15-Octadecatrienoic acid	C ₁₈ H ₃₀ O ₂	0.80 ± 0.02
11	9,12-Octadecadienoic acid	C ₁₈ H ₃₂ O ₂	7.38 ± 0.02
12	9-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	40.7 ± 0.3
13	12-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	2.50 ± 0.04
14	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	7.43 ± 0.05
15	Unidentified		0.04 ± 0.01
16	Nonadecanoic acid	C ₁₉ H ₃₈ O ₂	0.03 ± 0.01
17	11-Eicosenoic acid	C ₂₀ H ₃₈ O ₂	0.32 ± 0.04
18	Eicosanoic acid	C ₂₀ H ₄₀ O ₂	0.04 ± 0.01
	Saturated fatty acids		40.9 ± 0.4
	Unsaturated fatty acids		59.0 ± 0.6
	Total fatty acids		99.9 ± 0.9

ers are shown in Fig. 4. All results are expressed as means ± standard deviations. The results revealed that ostrich oil as vehicle significantly enhanced transdermal drug delivery of sinomenine. Among all the permeation enhancers evaluated, 2% Azone had the highest enhancing effect on the permeation of sinomenine through the rat skin. The penetration effect of ostrich oil on sinomenine was higher than other enhancers except 2% Azone. When 1% PG used as an enhancer in the control formulation, there was no promoting effect on the permeation of sinomenine. The result of blank control experiment had no difference compared with that of the control formulation containing 1% PG as permeation enhancer. The control formulation containing only 1% Azone existed lower enhancing effect on the permeation of sinomenine. Moreover, the combination of 1% Azone plus 1% PG used in the control formulation, the percutaneous permeation of sinomenine was obviously increased. The finding suggested that the enhancer activity of Azone may be increased by using cosolvents including PG²²⁾. As shown in Fig. 4, with the increase of Azone concentration from 1% to 2% (w/v), accumulative permeated amount of sinomenine increased, but decreased when Azone concentration reached to 3% (w/v). This result indicated that there was an optimum concentration for Azone

to achieve the best permeation-enhancing effect²³⁾. Beyond the apparent “optimum” Azone concentration (2% w/v), the permeation of sinomenine did not increase further.

The steady state flux of sinomenine obtained after permeation enhancers treatment is in the following decreasing order: 2% Azone > ostrich oil > 1% Azone + 1% PG > 1% Azone > 3% Azone ($p < 0.05$, Table 3). Moreover, it can also be clearly seen that ostrich oil exhibits better enhancement activity ($ER = 24.31$, $p < 0.05$). Ostrich oil has a high permeability coefficient (K_p) of 0.087 cm/h. The cumulative permeation amount of sinomenine in ostrich oil was $255.53 \pm 13.41 \mu\text{g}/\text{cm}^2$ after 24 h, which is almost 14.60-fold compared with 1% Azone as enhancer.

3.4 Fitting of permeation data to the mathematical models

Two mathematical models, Fick's law of diffusion and Scheuplein equation, which widely used to describe drug percutaneous permeation property, were applied to evaluate the drug diffusion mechanism (Table 3). The cumulative amount of drugs permeating through the skin was plotted as a function of time. The skin flux was determined from Fick's law of diffusion:

Fig. 3 HPLC chromatogram resulting from analysis of the effect of ostrich oil on permeation of sinomenine across rat skin (A) after 1 h (B) after 24 h.

Fig. 4 Permeation profiles of sinomenine through rat skin (mean $\pm$ S.D., $n = 4$).

$$J_{ss} = \frac{dQ_r}{Ad_t}$$

Where J_{ss} is the steady-state skin flux in $\mu\text{g}/\text{cm}^2/\text{h}$, dQ_r is the change in quantity of the drug passing through the skin into the receptor compartment in μg , A is the active diffusion area in cm^2 , and dt is the change in time. The steady-state flux (J_{ss}) was determined as the slope obtained by the linear regression of cumulative amounts of drugs (Q) against time (h)²⁴.

The permeability coefficient (K_p) was calculated as described in Scheuplein²⁵.

$$K_p = \frac{J_{ss}}{C_s}$$

Where C_s is the saturated solubility of sinomenine in

Table 3 Transdermal permeation data of sinomenine across rat abdominal skin.

Enhancers	J_{ss} , Flux ($\mu\text{g}/\text{cm}^2/\text{h}$)	K_p (cm/h)	T_{lag} (h)	ER
Ostrich oil	$10.01 \pm 1.36^*$	0.087^*	2.17	$24.31 \pm 1.29^*$
1% Azone	0.69 ± 0.17	0.006	3.26	1.51 ± 0.19
2% Azone	$12.56 \pm 1.69^*$	0.109^*	1.64	$29.97 \pm 1.53^*$
3% Azone	0.24 ± 0.11	0.002	2.45^*	0.54 ± 0.11
1% PG	0.11 ± 0.04	0.0009	4.37	0.14 ± 0.08
1%Azone + 1%PG	$5.30 \pm 0.62^*$	0.046^*	3.12	$11.29 \pm 0.73^*$

J_{ss} steady state drug flux through rat skin; T_{lag} the lag time in hrs; K_p Permeability coefficient; ER the enhancement ratios. Data are given as mean $\pm$ SD (n = 3)

* Statistically significant at $p < 0.05$.

donor solution. Different parameters viz. flux, permeability coefficient, diffusion coefficient, enhancement ratio, and lag time were calculated from the permeation data and the values are shown in Table 3.

4 CONCLUSION

Ostrich oil, a natural fat oil containing fatty acids, has been demonstrated to be more effective for enhancing the penetration of sinomenine across rat skin with significant permeation enhancement activity. It is well known that human skin is much different from rat skin, and ostrich oil may have different behavior in different skin models. Therefore, further studies are also needed in order to assess the performance of ostrich oil in a more authentic model such as human skin. In a word, with the characters like safety, non-irritancy, and non-toxicity to skin, ostrich oil can be potentially used in many pharmaceutical formulations as a moderately efficient, facile penetration vehicle, or used as a promising cosmetic adjuvant.

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ETHICAL CLEARANCE

Animal experiments were approved by Ethics Committee for Laboratory Animals Care and Use of Zhejiang University.

DECLARATION OF INTEREST

The authors have declared no conflict of interest.

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