Self-Microemulsifying Drug Delivery System (SMEDDS) of Vinpocetine: Formulation Development and in Vivo Assessment

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A new self-microemulsifying drug delivery system (SMEDDS) has been developed to increase the solubility, dissolution rate and oral bioavailability of vinpocetine (VIP), a poor water-soluble drug. The formulations of VIP-SMEDDS were optimized by solubility assay, compatibility tests, and pseudo-ternary phase diagrams analysis. The optimal ratio in the formulation of SMEDDS was found to be Labrafac : oleic acid : Cremophor EL : Transcutol P = 40 : 10 : 40 : 10 (w/w). The average particle diameter of VIP was less than 50 nm. In vitro dissolution study indicated that the dialysis method in reverse was better than the ultrafiltration method and the dialysis method in simulating the drug in vivo environment. Comparing with VIP crude drug power and commercial tablets, (−)-VIP-SMEDDS caused a 3.4- and 2.9-fold increase in the percent of accumulated dissolution at 3 h. Further study on the absorption property of VIP-SMEDDS employing in situ intestine of rats demonstrated that VIP in SMEDDS could be well-absorbed in general intestinal tract without specific absorption sites. In addition, the developed SMEDDS formulations significantly improved the oral bioavailability of VIP in rats. Relative bioavailability of (−)-VIP-SMEDDS and (+)-VIP-SMEDDS increased by 1.85- and 1.91-fold, respectively, in relative of VIP crude powder suspension. The mechanisms of enhanced bioavailability of VIP might contribute to the improved release, enhanced lymphatic transport, and increased intestinal permeability of the drug.

Key words vinpocetine; self-microemulsifying drug delivery system; formulation design; in vitro release; in situ absorption from intestine; bioavailability

Vinpocetine (VIP) is a semi-synthetic derivative of vincammine, an alkaloid derived from the plant Vinca minor L. belonging to the periwinkle family.3) VIP has several possible actions such as increasing cerebral blood flow and metabolism,2,3) anticonvulsant, cognition enhancement, neuroprotection1) and antioxidant.5) It is widely used in the treatment of chronic cerebral vascular ischemia,6) acute stroke,5) senile cerebral dysfunction,7) Alzheimer’s disease.8) VIP is virtually insoluble in water, and is cleared by extensive metabolism in the liver.9,10) VIP is mainly absorbed from the small intestine11) while the oral bioavailability is low. Under fasting condition, its oral bioavailability in human is as low as 7%, its clinical use is greatly restricted by the low oral bioavailability.13) However, the mechanism under the low bioavailability remains unknown. We hypothesis that the causes of its low bioavailability are as follows: Firstly, its poor water-solubility leads to poor release profile from formulation. Secondly, extensive metabolism in the liver could cause significant first-pass loss of active drug before it reaches systemic circulation. Thirdly, the single layer of intestinal epithelial cell might limit drug absorption/diffusion. It is worth noting that administration of VIP with food significantly increased its bioavailability,12) just because food can induce bile secretion and the drug is associated with mixed bile salt micelles in the small intestine to form a fine emulsion. Fine emulsion may enhance oral bioavailability by increasing the drug water-solubility and permeation, helping drug pass into the lymphatic vessels, and reducing liver metabolism.

To improve the solubility and bioavailability, self-microemulsifying drug delivery systems (SMEDDS) for VIP were developed. Self-emulsifying drug delivery systems (SEDDS) are defined as isotropic mixtures of oils, surfactants and co-solvents/surfactants.14,15) Upon mild agitation followed by dilution in aqueous media, such as GI fluids, the systems can form fine oil in water (o/w) emulsions with a droplet size between 100 and 1000 nm or microemulsions (SMEDDS) with a droplet size of less than 100 nm.16–18) The spontaneous formation of an emulsion/ microemulsion upon drug release presents the drug in a dissolved form and the small droplet size provides a large interfacial surface area favored for drug absorption. Drug exposure may be enhanced by the increased solubilization in gastrointestinal and enhanced accumulation in Peyer’s patch for lymphatic transport of the drug.19–23) Thus, for lipophilic drug such as vinpocetine (log Kow = 3.56<4),24) which is fit for formulations of SMEDDS,25) SMEDDS may offer an improvement in the rate and extent of absorption and a new strategy for enhancing oral bioavailability.

The objectives of this study were: 1) to develop and optimize the formulation of SMEDDS for vinpocetine; 2) to assess its properties in vitro; and 3) to evaluate its oral availability in vivo, using animal model.

MATERIALS AND METHODS

Reagents and Instruments Vinpocetine (VIP) fine powder was supplied by Nanjing Tianzun Zezong Chemical Co., Ltd. (China, Batch No: AET061095-000). Vinpocetine tablets were purchased from Northeast Pharmaceutical Co., Ltd. (Shenyang, China). Cremophor RH 40 and Cremophor EL were provided by BASF Co. (Germany). Isopropyl myristate (IPM) was obtained from Guangdong Yi Nong-hua
SMEDDS. The positively charged VIP-SMEDDS was ob-
room temperature for 48 h to obtain negatively charged VIP-
tate solubilization, shaken at 37 °C for 1 h and equilibrited at
then ultrasounded in a 37 °C water-bath for 20 min to facili-
diagrams. Following that, VIP was added. The mixture was
mixed by magnetic stirring at a certain ratio chosen from the
ous phase. The chosen oil and surfactant/cosurfactant were
water for visual observation of the self-emulsifying proper-
attack 500 ml of 37 °C water. The contents were then
release medium were put into the release medium for balance
emulsions was determined after 1 h by potentiometer and
dynamic light scattering. Viscosity was determined by vis-

Methods. Design of Formulations Solubility Assay: The solubility of VIP in various oils, surfactants, and cosur-
factants was first determined. An excess of VIP (approxi-
ately 200 mg) was dissolved in 2 ml of the indicated vehi-
cles in screw-capped glass vials. The mixture was heated at
40 °C in a shaking water-bath to facilitate solubilization. The
mixture was then shaken at room temperature for 48 h. After
reaching equilibrium, each vial was centrifuged at 4000 rpm
for 5 min. Insoluble VIP was then removed by filtration using
an oil membrane filter. The VIP in the supernatant was quan-
tified by HPLC as described below.

Compatibility Tests: To assess the compatibility, different
oils and surfactants, oils and surfactants at a ratio of (40 : 60)
were mixed for 5 min using a vortex mixer. Visual tests21,26) to
assess the self-emulsification properties of the blend were
also conducted. Fifty microliter (50 μl) of formulation was
pipetted into 50 ml of 37 °C water. The contents were then
mixed gently with a magnetic stir bar. The tendency of spon-
taneous emulsification and the progress of emulsion droplets
were observed. The performance of the formulations was vi-
sually assessed according to the grading system.22)

Pseudo-Ternary Phase Diagrams Study: The pseudo-ter-
nary phase diagrams were constructed to identify the self-
emulsifying regions and to identify the optimal composition
of formulation. The oil, surfactant and cosurfactant (total
0.2 ml) chosen from the compatibility tests were mixed evenly
at a serial of concentrations and were introduced into 300 ml
water for visual observation of the self-emulsifying proper-
ties.23) The microemulsion formulations at desired compo-
nent ratios were selected in combination of the identified mi-
icroemulsion region in the phase diagrams. The pseudo-ter-
nary phase diagrams of SMEDDS in the absence and pres-
ence of drug were also compared.

Preparation of SMEDDS SMEDDS formulations should be
kept clear after added sufficient drug and exposed to aque-
ous phase. The chosen oil and surfactant/cosurfactant were
mixed by magnetic stirring at a certain ratio chosen from the
diagrams. Following that, VIP was added. The mixture was
then ultrasounded in a 37 °C water-bath for 20 min to facili-
tate solubilization, shaken at 37 °C for 1 h and equilibrited at
room temperature for 48 h to obtain negatively charged VIP-
SMEDDS. The positively charged VIP-SMEDDS was ob-
tained by adding oleylamine.38)

Determination of the Physic-Chemical Properties

Emulsion Droplet Size, Zeta Potentiometer, and Viscosity
Analysis: SMEDDS (50 μl) was diluted with water (50 ml) in
a volumetric flask and gently mixed by inverting the flask.21)
The droplet size distribution and zeta potential of resultant
emulsions was determined after 1 h by potentiometer and
dynamic light scattering. Viscosity was determined by vis-

In Vitro Dissolution Test Dissolution study was per-
formed by three methods27,28): an ultrafiltration method, a
dialysis method, and a dialysis method in reverse. The ultra-
filtration method based on the ChP XC paddle method was
conducted as follows: an aliquot containing 5 mg of VIP
was introduced into 250 ml of enzyme-free simulated intesti-
tinal fluid (phosphate buffer saline, PBS 6.8) at 37 °C; and
the paddle revolution speed was set at 50 rpm. Two milliliters
of the solution was pipetted out at definite intervals, and fil-
tered through a membrane filter (0.22 μm). At the same time,
2 ml of blank release medium was added into the dissolution
apparatus. The concentration of VIP in the filtrate was meas-
ured by HPLC. The dialysis method29) was performed in sim-
ilar experimental conditions as follows: SMEDDS containing
5 mg of VIP was diluted in 10 ml release medium and then
instilled into dialysis bag (MWCO 10000, Spectrometer Medical
Industries Inc., U.S.A.). At planned time intervals, samples
from release medium were taken and analyzed. The third
method, the dialysis method in reverse was also studied. As
reported,28) about six dialysis bags containing 2 ml blank re-
lease medium were put into the release medium for balance
for 12 h, VIP-SMEDDS was then directly placed into the re-
lease medium instead of the bags. At each interval, one dial-
ysis bag was taken out and drug concentration in the bag was
determined. In vitro release of VIP, commercial tablets and
 crude drug powder were used as comparing controls. Be-
cause the food effect on VIP was significant,12) bile salts
might play a significant role in the dissolution and absorption
of VIP. The dissolution of VIP-SMEDDS and VIP in simu-
lated fed state medium containing 15 mm/l sodium tauro-
cholate, 3.75 mm/l lecithin, and pH 6.8 was also studied.

Absorption Property of Vinpocetine Self-Microemulsi-
Fying Drug Delivery System in Rat Intestine The absorp-
tion property of VIP-SMEDDS was conducted with the use
of in situ technique with intestine of rats. Experiments were
performed using established methods.29) Briefly, Sprague-
Dawley rats (male, 180—220 g) were fasted overnight before
the experiments. Anesthesia was induced with an i.p. injec-
tion of ethylcarbamate (15%, 5 ml/kg). The rats were then
placed on a warming pad under a surgical lamp to maintain
body temperature. A midline abdominal incision was made,
the intestinal segment of interest was located, and tubing was
inserted. The outlet tubing was placed 10 cm aboral to the
first opening. Using an infusion pump, the intestinal segment
was perfused at a flow rate of 1.0 ml/min with a 100 ml vol-
ume of circulating buffer containing VIP (20.0 μg/ml) and
phenol red (20 µg/ml), 1 ml sample was taken at time of 0, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0 h, 1 ml of blank circulating buffer containing phenol red (20 µg/ml) was added after each sampling. The concentration of VIP and phenol red was determined by HPLC and UV, respectively. The absorption constant (Ka) was calculated using Fink’s equation: $Ka = \frac{-\ln(X/X_0)}{t}$, where $X_0$ is the amount of drug before absorption, $X$ is the residue amount of drug after absorption. $Ka$ can be obtained as the slope from the regression curve of $-\ln(X/X_0)$ versus time. The influence of concentration of VIP and PH was also studied in rat’s jejunum segments where VIP crude powder was used as control. Because VIP is almost insoluble in water, 1.0% sodium dodecylsulphate (SDS) was added in the solution to help VIP dissolve.

**Determination of Vinpocetine in Rat Plasma by RP-HPLC** In this study, a modified HPLC/UV method was employed to determine VIP in rat plasma based on the method\(^{30}\) reported. The separation was achieved on a Europospher-100 C\(_18\) column (250 mm × 4.6 mm, 5 µm) at flow rate of 1.0 ml/min. The mobile phase was methanol: 0.1% triethylamine water solution (70:30), with pH adjusted by phosphoric acid to 5.5. A 50 µl amine water solution (70 : 30), with pH adjusted by phosphoric acid to 5.5. A 50 µl sample was injected into the column and the effluent was monitored at 274 nm. The column temperature was set to be 40°C.

Plasma extraction procedure was as follows: 1 ml plasma was added into a 10 ml screwcapped tube followed by 10 µl internal standard (11α-hydroxy-progesterone) solution (100 µg/ml) and 100 µl of sodium hydroxide (1 mol/l). After vortex mixing for 60 s, 5 ml ether was added and vortexed for 10 min. After centrifuging at 3000 g for 5 min, the organic layer was transferred to another tube and evaporated under a light stream of nitrogen at 37°C. The residual was dissolved by 100 µl methanol and 50 µl was injected for HPLC analysis.

The calibration curve was obtained by plotting the area ratios ($A_{vip} / A_{IS}$) against the concentration of VIP. Good linearity was observed over a concentration range of 50—3000 ng/ml, with the correlation equation being $R = -0.00164C$ (ng/ml) + 0.018 ($r = 0.998, n = 6$). Limit of quantification and limit of detection (S/N) was 50 and 10 ng/ml, respectively. At concentrations of 100, 1000, and 2000 ng/ml, extracted recoveries of VIP from rat plasma were 71.84%, 74.82%, and 76.23%; intra-day precision was 5.90%, 4.85%, and 9.88%, respectively. After storage for 1 month at −18°C and freeze-thawing for three times, VIP was stable in plasma. The above results showed that HPLC method was sensitive, precise and accurate for the determination of VIP.

**Bioavailability Studies** Male rats weighing 180—220 g were fasted for 12 h prior to the experiment. After oral gavage of a dose of VIP-SMEDDS (10 mg/kg, expressed as vinpocetine), about 2 ml of blood sample was collected at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0 h through eye sockets vein. Blood samples were centrifuged at 5000 g for 10 min, and plasma samples were separated and stored at −40°C.

Bioavailability of VIP-SMEDDS was compared with VIP tablets and crude drug powder suspension. Since VIP was virtually insoluble in water, VIP tablets or crude drug powder was mixed with 0.5% CMC-Na and supersoniced for 15 min to form a suspension containing 2.0 mg/ml of VIP. CMC-Na was used to help VIP stay in a good dispersed state.

### RESULTS AND DISCUSSION

**Solubility Studies** From the solubility results presented in Table 1, oleic acid, Labrafac, IPM, and olive oil were able to dissolve the drug and could be chosen as oils. Labrasol, cremophor EL and cremophor RH-40 could be chosen as surfactants. Transcutol P, a solubilizer and absorption enhancer, dissolved VIP efficiently, was then chosen as a cosurfactant.

**Compatibility Tests** The results of compatibility tests were summarized in Table 2. In comparison with formulations of long-chain glyceride such as oleic oil, IPM, and olive oil, formulations containing medium-chain glyceride such as LABRAFAC was found emulsifying better. Oleic oil has poorer emulsifying capability than LABRAFAC, which lead to better solubility than LABRAFAC in dissolving VIP. To prevent re-precipitation of drug when diluted by water, a polymeric precipitation such as SDS or hydroxypropyl methylcellulose (HPMC)\(^{22,31}\) was suggested to be added into the formulation to stabilize a temporarily supersaturated state of drug. But we found that it was not appropriate to add solid powder such as SDS or HPMC to a homogenous oil phase. So, a mixture of Labrafac and oleic oil at a ratio of 40:10 was recommended as the oil phase to improve the solubility. The use of stabilizers was avoided.

Labrasol was picked out from studied surfactants, because

| Table 1. Solubility of Vinpocetine in Various Vehicles (mg · ml\(^{-1}\), ± s) |
|-----------------|-----------------|-----------------|
| **Vehicles**    | **Solubility of** | **Vehicles**    | **Solubility of** |
|                 | **vinpocetine**  |                 | **vinpocetine**   |
| IPM             | 12.96            | Maisine35-1     | 3.40             |
| Labrafac        | 20.81            | Olive oil       | 10.60            |
| Coin oil        | 7.05             | Sesame oil      | 5.87             |
| Peanut oil      | 6.59             | Oleic acid      | 80.90            |
| Soybean oil     | 4.62             | Water           | 4.96 × 10\(^{-3}\) |
| Labrasol        | 33.19            | Cremophor RH 40 | 15.50            |
| Cremophor EL    | 18.90            | Tween 80        | 9.58             |
| Triton X-100    | 4.33             | Span 80         | 3.15             |
| Transcutol P    | 45.77            | Glycerin        | 6.64             |
| Propylene glycol| 8.75             | PEG400          | 5.67             |
| Alcohol         | 15.28            | Isopropanol     | 10.58            |

<table>
<thead>
<tr>
<th>Table 2. Results of Compatibility Tests</th>
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<tr>
<td><strong>Oil–surfactant</strong></td>
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<tr>
<td>-------------------</td>
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<tr>
<td>Oleic oil–Cremophor RH 40</td>
</tr>
<tr>
<td>Oleic oil–Cremophor EL</td>
</tr>
<tr>
<td>Oleic oil–Lab</td>
</tr>
<tr>
<td>Labrafac–Cremophor RH 40</td>
</tr>
<tr>
<td>Labrafac–Cremophor EL</td>
</tr>
<tr>
<td>Labrafac–Lab</td>
</tr>
<tr>
<td>IPM–Cremophor RH 40</td>
</tr>
<tr>
<td>IPM–Cremophor EL</td>
</tr>
<tr>
<td>IPM–Lab</td>
</tr>
<tr>
<td>Olive oil–Cremophor RH 40</td>
</tr>
<tr>
<td>Olive oil–Cremophor EL</td>
</tr>
<tr>
<td>Olive oil–Lab</td>
</tr>
</tbody>
</table>

\(^a\) Visual grading system: A, denoting a rapidly forming (within 1 min) microemulsion which was clear or slightly bluish in appearance; B, denoting a rapidly forming, slightly less clear microemulsion which had a bluish white appearance; C, denoting a bright white emulsion (similar to milk) that formed within 2 min; D, denoting a dull, grayish white emulsion with a slightly oily appearance that was slow to emulsify (longer than 2 min); E, denoting a formulation which exhibited either poor or minimal emulsification with large oil droplets present on the surface. "—" not detected.
its capability to emulsify was inferior to cremophor RH-40 and cremophor EL. Due to lower viscosity and similar capability of emulsifying as cremophor RH-40, cremophor EL was chosen as the surfactant. The desired component ratios were further investigated by the followed phase diagrams.

Compatibility tests were critical steps in the whole formulation design. The oils/surfactants with good solubility did not have good capability to emulsify. Through compatibility tests, it would be easy to know the characteristics and choose the optimal combination from the numerous oils and surfactants.

**Pseudo-ternary Phase Diagrams Study** A serials of SMEDDS was prepared and their self-emulsifying properties were observed visually.²¹) Pseudo-ternary phase diagrams were constructed to identify the self-emulsifying regions and to optimize the concentration of oil, surfactant (S) and cosurfactant (CoS). As shown in Fig. 1, efficiency of emulsification was good when the surfactant (Cremophor EL) concentration was more than 30% of formulation. It was observed that increasing concentration of the cosurfactant (Transcutol P) within the self-emulsifying region caused increased spontaneity of the self-emulsification process. When a cosurfactant was added to the system, it further lowered the interfacial tension between the oil and water interface and also influenced the interfacial film curvature and stability. On the other hand, factors of safety should be considered with the increasing concentration of S and CoS. Therefore, on the basis of using small amount of surfactant and cosurfactant, the optimal ratio in the formulation of SMEDDS was Labrafac : oleic acid : Cremophor EL : Transcutol P = 40 : 10 : 40 : 10 (w/w). There were almost no differences between the phase diagrams in the absence and presence of VIP. As shown in Fig. 1, something interesting was that the self-emulsified region of mixture of oils became larger than that of either oil alone. The possible reason may be that the polarity of mixed oil had been changed and the molecule of oil was easy to enter the surfactant, stable drops of emulsion formed with the decreased surface tension. The compositions of formulations containing VIP were given in Table 3. The selected excipients used in the formulations have all been approved for drug use by U.S.FDA. Due to the relatively high concentration of surfactant in formulations, the acute toxicity of VIP-SMEDDS was studied. The LD₅₀ of (-)VIP-SMEDDS and (+)VIP-SMEDDS was both 20.2 g/kg for rats (oral, 7 d follow-up period), suggesting the formulations of VIP-SMEDDS had high tolerance and low toxicity in rats.

**Physic-chemical Characteristics of SMEDDS** The results of emulsion droplet size, zeta potential, and viscosity analysis were shown in Table 3. The shape and surface morphology of microemulsion were shown in Fig. 2. It was observed that droplet of microemulsion was almost of spherical shape with smooth surface and the drug mainly dispersed in the hydrophobic core consisted of oil phase and surfactant. The droplet size of (+)VIP-SMEDDS was a little bigger than that of (-)VIP-SMEDDS.

**In Vitro Dissolution Study** According to studies presented in Fig. 3, the dissolution of VIP-SMEDDS varied when using different methods. Although the three methods were usually used to detect dissolution of microsphere and microemulsion, but there were no reports comparing dissolution of SMEDDS using the three methods together. The rank of the dissolution value of VIP-SMEDDS was ultrafiltration method > dialysis method in reverse > dialysis method. The ultrafiltration method was the simplest method to operate, in

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**Table 3. Formulations and Characteristics of SMEDDS**

<table>
<thead>
<tr>
<th>Items</th>
<th>(-)VIP-SMEDDS</th>
<th>(+)VIP-SMEDDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulations</td>
<td>Labrafac 40</td>
<td>Labrafac 40</td>
</tr>
<tr>
<td></td>
<td>Oleic acid 10</td>
<td>Oleic acid 10</td>
</tr>
<tr>
<td></td>
<td>Cremophor EL 40</td>
<td>Cremophor EL 40</td>
</tr>
<tr>
<td></td>
<td>Transcutol P 10</td>
<td>Transcutol P 10</td>
</tr>
<tr>
<td>VIP</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Oleylamine</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Droplet size (nm)</td>
<td>37.59±4.28</td>
<td>40.18±4.85</td>
</tr>
<tr>
<td>ζ-Potential (mV)</td>
<td>-26.64±2.34</td>
<td>+6.24±1.46</td>
</tr>
<tr>
<td>Viscosity (mPa·s)</td>
<td>219.7±13.6</td>
<td>211.4±9.8</td>
</tr>
</tbody>
</table>

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Fig. 1. Pseudo-ternary Phase Diagram of SMEDDS
which the drug was diluted with enough media. The results of dissolution represented the total amount of free drug molecules and the drug in microemulsion with the diameter below 220 nm (diameter of filter membrane). To some extent, it could represent the release of VIP-SMEDDS in the in vivo circumstance. In the dialysis method, the drug was diluted with 10 ml media, the diffusion process of drug was slow because of the low concentration grade and the barrier of dialysis. The dialysis method in reverse was the most suitable method that better simulated the circumstance of drug in vivo. The microemulsion of VIP was more readily formed and the diffusion process of drug became quicker due to the large concentration grade. Similarly, the barrier of dialysis might slow the process. The result of dissolution by the dialysis method in reverse was the total amount of free drug molecules, excluding the drug in microemulsion.

Figure 4 showed the dissolution results of VIP-SMEDDS by the dialysis method in reverse compared with VIP tablets and crude drug powder. The percent of accumulated dissolution of VIP in SMEDDS in PBS 6.8 at 3 h was up to 68.45%, which was 3.4 times as much as that of VIP crude powder (20.01%) and 2.9 times of commercial tablets (23.55%). The dissolution results of VIP-SMEDDS by ultrafiltration method were shown in Fig. 5. The percent of accumulated dissolution of VIP-SMEDDS at 3 h in PBS 6.8 was up to 92.55%, a 7.7-fold increase comparing with VIP crude powder (11.96%) and 4.2-fold increase comparing with commercial tablets (21.88%). Different zeta potential of SMEDDS almost had no effects on the dissolution. By ultrafiltration method, the dissolution of VIP in media simulating fed state conditions was apparently approved, which showed bile salt (an endogenous surfactant) played an important role in the improvement of dissolution and absorption of VIP. But the percent of accumulated dissolution of VIP-SMEDDS in the simulated fed state media was similar to that in enzyme-free simulated intestinal fluid. In other words, food would have minimal effects on the dissolution and absorption of VIP. Moreover, there was no precipitation and stratification when VIP-SMEDDS dissolved in the simulated fed state media.

Absorption Property of VIP-SMEDDS in Rat Intestine
The absorption constant (Ka) of VIP-SMEDDS at the ileum, jejunum, duodenum and colon were 0.247, 0.273, 0.259 and 0.226 h⁻¹, respectively. There were not apparent differences between them. Unlike the reported on VIP in hydroxypropyl-β-cyclodextrin, value of Ka at colon was significantly
Of VIP tablet and crude drug powder suspension. The curve SMEDDS were significantly higher than those treated with VIP plasma concentrations in rats treated with VIP-drug powder suspension. At all the indicated time points, concentration–time curves in rats after a single oral dose of SMEDDS, VIP-tablet and crude powder were as follows: Table 5. Mean parameters for Table 5. The Pharmacokinetic Parameters of VIP in Rats after Oral Administration by Dose (10 mg/kg) of Different Preparations (\(n=5\))

<table>
<thead>
<tr>
<th>Parameters</th>
<th>VIP-SMEDDS</th>
<th>(+)VIP-SMEDDS</th>
<th>VIP-Tablets</th>
<th>VIP-Powder</th>
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<tr>
<td>(MRT_{\text{je}}) (h)</td>
<td>2.96±0.82</td>
<td>2.83±0.68</td>
<td>2.45±0.56</td>
<td>2.44±0.50</td>
</tr>
<tr>
<td>(C_{\text{max}}) ((\mu)g/ml)</td>
<td>0.98±0.26</td>
<td>1.04±0.30</td>
<td>0.72±0.21</td>
<td>0.70±0.25</td>
</tr>
<tr>
<td>(T_{\text{max}}) (h)</td>
<td>1.48±0.39</td>
<td>1.43±0.24</td>
<td>1.02±0.13</td>
<td>1.01±0.22</td>
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<tr>
<td>(t_{\text{1/2}}) (h)</td>
<td>2.62±0.32</td>
<td>2.48±0.49</td>
<td>1.79±0.11</td>
<td>1.55±0.59</td>
</tr>
<tr>
<td>(CL) (l/h/kg)</td>
<td>2.22±0.59</td>
<td>2.03±0.76</td>
<td>3.98±0.28</td>
<td>4.34±0.63</td>
</tr>
<tr>
<td>(AUC_{\text{0-8h}}) ((\mu)g·min/ml)</td>
<td>258.15±30.12</td>
<td>267.57±33.56</td>
<td>144.72±20.74</td>
<td>139.92±26.86</td>
</tr>
<tr>
<td>(F_{i})</td>
<td>1.84±0.62</td>
<td>1.91±0.48</td>
<td>1.03±0.23</td>
<td>—</td>
</tr>
</tbody>
</table>

\(a\) The absorption parameters of (-)VIP-SMEDDS or VIP of different concentrations at \(pH\) 6.8. \(b\) The absorption parameters of (-)VIP-SMEDDS or VIP of 20.0 \(\mu\)g·ml\(^{-1}\) concentration at different \(pH\). Table 5. The Pharmacokinetic Parameters of VIP in Rats after Oral Administration by Dose (10 mg/kg) of Different Preparations (\(n=6\), \(\bar{x}\)±s)

lower than other rat’s intestine segments. The results summarized in Table 4 suggested that \(K_{a}\) of VIP-SMEDDS in jejunum was not apparently influenced by different concentration of VIP and \(pH\), and SMEDDS could significantly enhance the absorption of VIP without specific absorption section in small intestine and colon.

**Bioavailability Study** Figure 6 showed the plasma concentration–time curves in rats after a single oral dose of VIP-SMEDDS in comparing with VIP tablets and crude drug powder suspension. At all the indicated time points, the VIP plasma concentrations in rats treated with VIP-SMEDDS were significantly higher than those treated with VIP tablet and crude drug powder suspension. The curve of (+)VIP-SMEDDS was also found a little higher than (-)VIP-SMEDDS. Ten hours after oral administration, the VIP plasma concentrations were below 50 ng/ml, whereas the drug was undetectable 6 h after administration of VIP tablet and suspension.

The oral pharmacokinetic parameters of rats were listed in Table 5. Mean parameters for (-)VIP-SMEDDS, (+)VIP-SMEDDS, VIP-tablet and crude powder were as follows: \(C_{\text{max}}\): (0.98±0.26), (1.04±0.30), (0.72±0.21) and (0.70±0.25) \(\mu\)g/ml; \(T_{\text{max}}\): (1.48±0.39), (1.43±0.24), (1.02±0.13) and (1.01±0.22); \(AUC_{\text{0-8h}}\): (258.15±30.12), (267.57±33.56), (144.72±20.74) and (139.92±26.86) \(\mu\)g·min/ml. In comparing with VIP crude powder, the mean relative bioavailability of (-)VIP-SMEDDS and (+)VIP-SMEDDS increased by 1.85- and 1.91-fold, respectively, while the bioavailability of VIP tablet was almost equivalent to that of VIP crude powder. Drug exposure of the positively charged SMEDDS was a little higher than the negatively charged SMEDDS. Owing to the negatively charged character of intestinal mucous membrane, the positively charged SMEDDS had tighter adhesion than the negatively charged formulation to the cell surface due to the electrostatic attraction. In addition, the markedly decreased clearance value in SMEDDS in relative of tablets and drug powder might be explained by improved lymphatic transport pathway, reduced metabolism in the liver, and possible lipid protection of drug from enzymatic degradation.

The possible reasons of low bioavailability of VIP were its poor water-solubility, extensive metabolism in the liver, and the barrier of the single layer of intestinal epithelial cell. In our dissolution study in vitro, it was confirmed that SMEDDS offered an improvement in the rate and extent of
dissolution of VIP. In the absorption study in rat intestine, the results suggested that SMEDDS could markedly promote the absorption of VIP in general intestinal tract, including colon. The fine microemulsion might not only increase the water-solubility of drug, but also enhance accumulation in Peyer's patch for lymphatic transport of the drug, thus avoid being metabolized by the liver. The formed oil droplets also stimulated lipoprotein/chylomicron production, an effect similar to food that could apparently enhance the bioavailability of VIP.\(^\text{[12]}\)

High content of surfactants used in formulations of SMEDDS are known to play an important role in improving the bioavailability by various mechanisms including: 1. improved drug dissolution\(^\text{[35]}\), 2. increased intestinal epithelial permeability by disturbing the cell membrane.\(^\text{[21,36]}\) Surfactant monomers are capable of partitioning into the cell membrane where they can form polar defects in the lipid bilayer. At high surfactant concentrations in the cell membrane, the membrane could be dissolved into surfactant–membrane mixed micelles\(^\text{[3,13,6]}\), 3. increased a reversible effect on the opening of tight junction permeability.\(^\text{[37,38]}\) SMEDDS may interact with the polar head groups of the lipid bilayers, modifying hydrogen bonding and ionic forces between these groups. It may also insert itself between the lipophilic tails of the bilayers, resulting in a disruption of the lipid-packing arrangement. In our study of absorption of VIP in Caco-2 cells, which would be further reported in details separately, the tight junction of small intestine could be widely opened by VIP-SMEDDS, comparing with VIP crude powder; 4. decreased/inhibited \(p\)-glycoprotein drug efflux.\(^\text{[39]}\) VIP is metabolized by phosphodiesterase, and is not the substrate of \(p\)-glycoprotein, so the enhancement of absorption of VIP is not modified by this mechanism.

CONCLUSIONS

Taken together, a SMEDDS formulation was developed and the optimal ratio in the formulation was Labrafil\(c\) : oleic acid : Cremophor EL : Transcutol P = 40 : 10 : 40 : 10 (w/w). When diluted with water, \((-)\)VIP-SMEDDS could spontaneously form small particles with average particle size of \((37.59\pm4.28)\) nm. In vitro dissolution studies demonstrated that the dialysis method in reverse was better than both the ultrafiltration method and the dialysis method in simulating the \(in\ vivo\) circumstance of drug. The percent of accumulated dissolution of vinpocetine at 3 h in PBS 6.8 was up to 68.45%, representing a 3.4-fold and 2.9-fold increase in relative of VIP crude powder and commercial tablets, respectively. The absorption property of VIP-SMEDDS was also studied with the use of \(in\ situ\) rat’s intestine technique. VIP in SMEDDS could be absorbed well in general intestinal tract without specific absorption sites. Relative bioavailability of \((-)\)VIP-SMEDDS and \((+)\)VIP-SMEDDS was 1.85- and 1.91-fold that of VIP crude powder suspension, while the bioavailability of VIP tablet was almost equivalent to that of VIP crude powder. The mechanisms on enhancement of bioavailability of vinpocetine might attribute from the improved release, the enhanced accumulation for lymphatic transport, and the increased intestinal permeability of the drug.

Our studies highlighted the potential of using SMEDDS as efficient strategy for the oral delivery of hydrophobic compounds such as vinpocetine. Since nearly 40% of drug compounds are hydrophobic, SMEDDS is a promising formulation approach for improving oral bioavailability of drug with poor aqueous solubility.\(^\text{[14]}\) It appears to be a trend that more drug products will be formulated as SMEDDS in the very near future. The efficiency of the SMEDDS formulation is drug-dependent in most instances, thus the successful composition of the SMEDDS formulation should be carefully explored.

REFERENCES AND NOTES