Thienorphine hydrochloride (ThH) is a highly insoluble and readily metabolized partial-opioid agonist. It is used for the treatment of pain and heroin addiction. This study aimed to formulate and evaluate sublingual delivery systems containing ThH. Dimethyl-β-cyclodextrin (DM-β-CD) can enhance the solubility and permeability of hydrophobic drugs. In this paper, ThH cyclodextrin inclusion complexes were prepared and administrated sublingually with the objective of improving the drug’s aqueous solubility, in vitro permeation rate, and in vivo absorption rate. The formulation was prepared with DM-β-CD using the freeze-dried method and characterized using phase solubility, differential scanning calorimetry (DSC), X-ray and NMR analyses. The results of each test indicated the formation of dynamic inclusion complexes between ThH and DM-β-CD. The inclusion complexes also showed significant increases in in vitro aqueous solubility and mucosal permeability. According to the pharmacokinetic study of the complex in rats, the AUC and Cmax values of the sublingual delivery group were 40 and 46 times higher than those of the gastrointestinal group, whereas tmax was shorter, which proved that in vivo absorption and metabolism had been improved. It can therefore be concluded that the inclusion technology and sublingual delivery system were suitable for ThH development.

Key words bioavailability; sublingual drug delivery; absorption; solubility; cyclodextrin; thienorphine

Thienorphine (21-cyclopropyl-7-[1-(R)-1-hydroxy-1-methyl-3-(thien-2-yl)propyl]-6,14-endothano-6,7,8,14-tetrahydro-oripavine, Fig. 1, is a new type of partial-opioid agonist, was synthesized by the chemists in our institute. Meanwhile, ThH, a hydrochloride salt with a crystal water of thienorphine, has been developed as drug candidate. It has fared comparatively well with respect to aqueous solubility and pharmacodynamic action compared to its parent compound and its other salts. The drugs currently in clinical use for the treatment of opioid dependence are either full-opioid agonists or antagonists. Partial-opioid agonists could be more widely used because they provide certain advantages. They have unique pharmacological properties that allow them to prevent the addiction that can occur with full-opioid agonists and there has been a lack of subjective side effects. Although ThH tablets have already passed through clinical testing in China, further development of ThH has been restricted by its poor solubility, which causes a great deal of dissolution in the gastrointestinal (GI) tract and significant enteric excretion. Second, it is extensively metabolized by the liver, as previously indicated.

Oral mucosal drug delivery is an alternative dosage form for thienorphine. It has been suggested that the sublingual route has the following advantages: sufficient blood supply, avoidance of the liver and GI, rapid absorption, and high bioavailability. Buccal and sublingual sectors are commonly used routes for drug delivery. The sublingual is more permeable and thinner than the buccal mucosa, with higher blood flow. This suggests that one way of improving ThH bioavailability may be the development of a sublingual delivery system. However, sublingual delivery has strict requirements. The compound must first dissolve and disperse into the saliva. Then it must cross the unstirred water layer consisting of the mucin network and finally the oral membrane. In addition, permeation across the sublingual mucosa can be easily achieved when the concentration of active ingredient is relatively high. Therefore, to develop a preparation of ThH suitable for sublingual delivery, increasing solubility is the primary concern.

Cyclodextrins (CDs), cyclic oligosaccharides consisting of 6–8 glucopyranose units, can trap the lipophilic drug in a cage-like meshwork. So far, CDs have been reported to increase the solubility, stability, and bioavailability of drugs in a few different delivery systems. Many studies have reported that β-cyclodextrin (β-CD, Fig. 2) is a good natural cyclodextrin because of its efficient drug complexation and availability in pure form. However, its low water solubility (1.5×10−2 m) and toxicity limit their application in pharmaceutical formulations. Recently, various kinds of chemically modified CD derivatives have been prepared to extend the physicochemical properties and inclusion capacity of parent CDs. One of the derivatives is dimethyl-β-cyclodextrin (DM-β-CD, Fig. 2), which has more pronounced solubilization (0.2 m) and complexation than its parent compound. It also has some features that make it safer for use in clinical settings. The DM-β-CD in particular has been shown to be excellent absorption enhancers in mucosal drug delivery. These all indicate that they are well suited to sublingual administration.

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delivery system for ThH-DM-β-CD inclusion complexes. Based on the in vitro results, a suitable formulation was constructed and administered to test rats by a sublingual route. Pharmacokinetic parameters were compared to those of rats given the drug via the gastrointestinal tract (GIT). The results of each test indicate that the inclusion complexes prepared by the co-lyophilization method were satisfactory with respect to complexing and increasing solubility, permeability, and bioavailability.

Experimental

Materials ThH (>99%) was synthesized in the Institute of Pharmacology and Toxicology (Beijing, China). Buprenorphine standard (>99%) n-octanol (>99%) was purchased from the Sigma-Aldrich Chemical Co. (U.S.A.). DM-β-CD (>98%) was purchased from the Li Quan Co., Ltd. (Shanxi, China). Methanol, acetonitrile and other materials were of analytical grade.

Preparation of ThH-DM-β-CD Complexes The bulk ThH-DM-β-CD complexes were prepared according to the freeze-dried method as follows: ThH and the corresponding amount of DM-β-CD (molar ratio 1:2) were dissolved in methanol and water, respectively. The resulting aqueous mixtures were stirred for 2 h at constant temperature of 60°C. The above preparation was then frozen and freeze-dried for 24 h, using a lyophilizer (MDF-382E, Sihuan, China) at −80°C for 24 h.

Aqueous Solubility The aqueous solubility of the ThH, ThH/DM-β-CD physical mixture and its DM-β-CD complexes were compared using a constant-temperature shaker (THX-82, Shanghai, China). The suspensions with excess amounts of ThH, physical mixture, and inclusion complex were placed in capped tubes. The tubes were placed in a water bath at a constant temperature (37°C), and were shaken in horizontal movements (100 min⁻¹) for 48 h to allow equilibrium. After equilibrium was attained, an aliquot was filtered through a 0.45 µm Millipore filter, and the thienorphine content was assayed by HPLC.

Partition Coefficient Water and oil solutions (n-octanol) were saturated with each other before the experiment. Solutions of ThH and ThH/DM-β-CD physical mixture (molar ratio 1:2) and ThH-DM-β-CD complexes (100 µg/mL) were prepared with water saturated with oil phase. One milliliter of each solution was then transferred to 10 mL centrifuge tubes containing 1 mL of oil phase saturated with water. The tubes were vortex-mixed for 2 h at 37°C and centrifuged at 3000 × g for 5 min. After centrifugation, 100 µL was withdrawn from the water phase and assayed by HPLC at time zero (C₀) and after shaking to ensure partition (C₅₀). The partition coefficient was K_{oil/water} = (C₅₀ - C₀)/C₀. The experiments were performed in triplicate.

HPLC analysis of thienorphine was performed on a Shimadzu 10A HPLC system (Shimadzu, Japan). The detection wavelength was set at 220 nm. Chromatographic separation was carried out with a Varian ODS-C18 column (4.6 mm × 250 mm, 5 µm; Varian, U.S.A.) with mobile phase consisted of methanol and phosphoric acid solution (pH=3) in a ratio of 40:60 (v/v), and the column temperature was 35°C. The flow rate was 1.0 mL·min⁻¹.

Phase Solubility Test The phase solubility studies were performed according to the method of Higuchi and Connors. A schematic explanation of the principle of this technique is given below, via Eq. 1:

$$K_s \left( \frac{C_{s}}{K_d} \right) = \text{ThH:DM-β-CD}$$

Here Kₛ is the combination rate constant for the complex formation and K_d is the dissociation rate constant of the complex.

Excess ThH was added into aqueous solutions of DM-β-CD at a serial concentrations in capped tubes and shaken at 25°C for 48 h. After reaching equilibrium, the suspensions were filtered through a 0.45 µm Millipore filter followed by the quantification of the drug by HPLC. Phase solubility curves were represented as the total dissolved drug concentration relative to the concentration of DM-β-CD. The stable constant (K_s) was calculated from the initial straight portion of the phase solubility diagram using Eq. 2:

$$K_s = \frac{\text{slope}}{S_0(1 - \text{slope})}$$

Here S₀ is the solubility of ThH in the absence of DM-β-CD (equal to the intercept of the diagram), slope is the slope of the experimental phase solubility diagram for ThH-DM-β-CD, and K_s is the stability constant (K_s/K₅). The phase solubility data were plotted as ThH vs. DM-β-CD.

Differential Scanning Calorimetry (DSC) Test Thermal properties of the powder samples were investigated with a differential scanning calorimeter (CDR-4P, Shanghai, China). Approximately 10 mg of sample was analyzed in an open aluminum pan, and heated at a scanning rate of 10°C·min⁻¹ from room temperature to 500°C. The results indicated the differences in the profiles, which were attributed to ThH, DM-β-CD, the physical mixture, and the ThH-DM-β-CD complex.

X-Ray Diffractometry (XRD) Test Diffraction patterns were obtained using a high resolution X-ray diffractometer (D/max r-B, Rigaku, Japan), using Ni-filtered CuKa (1.542 Å) radiation (40 kV, 40 mA). Powder samples were mounted on a sample holder and scanned from 5° to 50° in 2θ at a speed of 0.02°·min⁻¹. The samples analyzed were the same as those used in the DSC experiments.

Proton Nuclear Magnetic Resonance (NMR) Spectra Test All proton detection experiments including one-dimensional ¹H-NMR (400 MHz) and the nuclear Overhauser effect
spectroscopy (NOESY) of the sample in deuterium oxide (D₂O) were conducted using a JNM-ECA-400C spectrometer operated at 25°C. To eliminate the interference of moisture, all the samples and NMR tubes were vacuum dried at 80°C over 6 h before analysis. The chemical shifts are expressed using tetramethylsilane as an internal standard.

In Vitro Mucosa Permeation Test The permeability study was carried out with Franz diffusion cells. The fresh porcine sublingual mucosa was used as a model in this test, owing to the similarity of composition, structure and permeability measurements between pigs and humans. Sublingual mucosal specimens were obtained from tissue removed from freshly slaughtered pigs and equilibrated in phosphate buffered saline (PBS) for 1 h before starting experiments. The Franz diffusion cells had a receiver volume of 6.5 mL and diffusional area of 0.7 cm². The receiver compartments were filled with degassed dissolution medium, which was a mixture ethanol and PBS at pH 6.8 (2:3, v/v), to promote drug solubilization in the receptor. This medium did not alter barrier permeability. Micromagnetic stirrer bars were added to the receptor compartments and the complete cells were placed in a water bath at 37°C. The sample (drug or drug-CD system equivalent to 0.8 mg of ThH) was applied over the mucosa surface. The donor compartment was closed with the screw cap to preserve the humid environment of the mucosa. At predetermined times, aliquots of the receptor medium were removed and replaced with fresh dissolution medium. The samples withdrawn from this system were diluted and analyzed by HPLC. The HPLC conditions were the same as those used in the solubility test. Each test was performed eight times.

The flux values (Jₚ, µg·cm⁻²·min⁻¹) across the mucosa were calculated at the steady state per unit area by linear regression analysis of permeation data, as shown in Eq. 3:

\[ J_p = \frac{Q}{A} \]  

(3)

Here Qᵣ is the quantity of ThH which passes through the tissue into the receptor compartment (µg), A is the orifice area for diffusion (cm²), and t is the duration of exposure (min).

The permeability coefficient (Kₚ, cm·min⁻¹) was then calculated via Eq. 4:

\[ K_p = \frac{J_p}{C_d} \]  

(4)

Here Jₛ is the flux calculated at the steady state (µg·cm⁻²·min⁻¹), and C_d is the drug concentration in the donor compartment (400 µg·cm⁻³). Inclusion complexes displayed much more aqueous solubility, which was 10469.08 µg·mL⁻¹.

In Vivo Absorption Test. Animals Wistar rats (200±20 g) supplied by the Academy of Military Medical Sciences Animals Center (Beijing, China) were used for pharmacokinetic studies. The animal were acclimatized at a temperature of 25±2°C and a relative humidity of 70±5% under natural light/dark conditions for 1 week and were given food and water ad libitum. Prior to the experiment, the animals were kept under fasting conditions overnight. All experimental procedures abided by the ethics and regulation of animal procedures abided by the ethics and regulation of animal experiments of the Academy of Military Medical Sciences, China.

Pharmacokinetic Test The dose of ThH-DM-β-CD complex solution GI delivery; II: ThH-DM-β-CD complex solution sublingual delivery). When administered sublingually, rats were anesthetized with halothane (5% induction, 0.7% maintenance dose), and a suitable volume of liquid formulation was administered underneath the tongue using a pipette. The lower jaws of the rats were supported in the horizontal position for 20 min to minimize the chance of swallowing. Serial blood samples were collected at predetermined points in time (at 5, 10, 20, 30, 40 min and 1, 2, 3, 4, 7, 12, 24 h after GI administration; at 2, 5, 10, 20, 40 min and 1, 2, 3, 4, 7, 12, 24 h after sublingual administration). Then 0.3 mL blood samples were collected, and the sample was centrifuged (3000×g, 15 min) and plasma was collected and stored at −20°C until analysis. The methods of plasma sample preparation and detection were identical to those used by Kong et al. The concentration of ThH was quantitated in this test.

Statistics Statistical differences were estimated using student’s t-test. Pharmacokinetic parameters were obtained using the practical pharmacokinetic program version 87 (Committee of Mathematic Pharmacology of the Chinese Society of Pharmacology, China).

Results and Discussion

Solubility Test The solubility of bulk ThH was only 479.23 µg·mL⁻¹ at 37°C. As shown in Fig. 3. The physical mixture (ThH:DM-β-CD=1:4, mole ratio) showed a slightly increase in the solubility of ThH (903.77 µg·mL⁻¹). The inclusion complexes displayed much more aqueous solubility, which was 10469.08 µg·mL⁻¹.

Cyclodextrins are useful functional excipients that have enjoyed widespread attention and use. The reason for this popularity is the ability of these materials to interact with poorly water-soluble drugs and drug candidates, increasing their apparent aqueous solubility. ThH is a highly insoluble, highly metabolized drug candidate. It was solubilized by DM-β-CD via formation of a soluble complex. The characterization results of the complex indicate that ThH is amorphous and consistent with inclusion of the ThH in the DM-β-CD cavity. The fact that both ThH and DM-β-CD are both amorphous lead to an enhanced solubility, and DM-β-CD stabilizes the ThH thereby preventing crystallization. The significant difference between the saturation solubility of the physical mixture and that of the lyophilized complex indicated that the preparation process is vital for this combination.
Partition Coefficient Several solvent systems have been used to relate partition coefficients to mucosal absorption. N-Octanol is a suitable system because its polar and non-polar nature mimics the complex nature of the mucosa. In this study, the partition coefficient of DM-β-CD complexes (K_{oil-water}≈13) was found to be approximately half that of the pure drug (K_{oil-water}≈26). This showed that the lipophilic characteristics of ThH decreased when it was complexed with CDs. This result was not same as the experiment by Frijlink, which showed that the addition of β-CD was not due to the obvious decrease in partition coefficients. This result was consistent with another experiment, which showed that the partition coefficients of pure drugs decreased when those drugs were complexed with various CDs. Partition coefficients of papaverine alone or in the presence of DM-β-CD at pH 7.4 was tested, and the value was obviously decreases in the complexes. We insist that the difference between those results was caused by the absence of drug-cyclodextrin interactions. In the solubility test, the physical mixture showed a slight increase in the solubility of ThH, while the inclusion complexes displayed much higher aqueous solubility.

Lipophilicity is one of the determinants of drug permeability through membranes, and the partition coefficient is closely related to the lipophilicity. However, lower lipophilicity does not equal to poor ability to cross the sublingual mucosa. The drug transport mechanism through the mucosa involves two major routes: the transcellular (across the cells) and paracellular (between the cells) pathways. The presence of tight junctions between intestinal epithelial cells is the primary barrier to paracellular drug transport through the intestine and nasal mucosa. However, tight junctions are rare in the oral mucosa. Therefore, most compounds actually traverse the buccal mucosa via the paracellular route. The increase in aqueous solubility and decrease in lipophilicity of ThH with inclusion may not have led to poor ability to cross the sublingual mucosa.

Phase Solubility Test Phase solubility testing of the effect of complexing agents on the compound being solubilized is a standard approach to determining not only the value of the stability constant but also to finding insight into the stoichiometry of the equilibrium. The 1:1 drug–cyclodextrin complex is the most common type of association. In this association, a single drug molecule is included in the cavity of one cyclodextrin molecule, and there is a stability constant for the association, a single drug molecule is included in the cavity of one cyclodextrin molecule. This in turn led to an almost complete loss of crystallinity in this binary system.

Differential Scanning Calorimetry Test DSC is used in the pharmaceutical field to establish the identity and purity of solid-state systems and to detect interactions between their components. Incorporation of a guest molecule into the CD cavity can cause its melting, boiling, and sublimation points to shift or disappear. Figure 5 shows DSC thermograms of the pure components, the physical mixture, and lyophilized powder of ThH-DM-β-CD complexes. The curve of ThH (Fig. 5A) displayed two endothermic peaks: a broad endotherm between 80°C and 120°C corresponds to the loss of bounded water. The other is a sharp endothermic peak at 258.16°C, indicating the melting point. The thermogram of pure DM-β-CD (Fig. 5B) powder exhibited a broad endothermic peak near 100°C. This is because β-CD did not produce any peaks of interest when examined by DSC. This result was consistent with those of other reports. The physical mixture of ThH and DM-β-CD (Fig. 5C) apparently contains only the free species, but they are present at a much lower level of intensity due to the dilution by DM-β-CD. This indicated the absence of drug-cyclodextrin interactions. A different pattern was observed in the thermogram of the association complexes (Fig. 5D). No appreciable endothermic event associated with the melting of the ThH was observed, suggesting the likelihood of formation of true inclusion complexes between ThH molecules and DM-β-CD. This in turn led to an almost complete loss of crystallinity in this binary system.

Fig. 4. Phase Solubility Profile of ThH with DM-β-CD in Distilled Water at 25°C (n=3, Mean±S.D.)
namely a-H, b-H, c-H (thiophene protons) as well as d-H (oripavine protons). The proton signals of ThH are affected by the inclusion process and the change of chemical shifts of various relevant protons in the ThH is listed in Table 1. It is seen that the chemical shift changes ($\Delta\delta$) of the c-H are larger compared with d-H. On the whole, the change of chemical shifts showed the following order: $\Delta\delta$ c-H > $\Delta\delta$ b-H > $\Delta\delta$ a-H > $\Delta\delta$ d-H. It seems reasonable to postulate that the thiophene ring is deeply included inside the CD cavity. In addition, it can be seen after inclusion, there were slightly downfield chemical shift of the protons (3-H, 5-H) in the CD cavity. The results indicated that the environment of these protons was changed after the inclusion of ThH with the DM-\(\beta\)-CD.

**2D-NMR test** For a better understanding of the interaction model of the inclusion complex of ThH-DM-\(\beta\)-CD, 2D-NMR spectroscopy has also been used to study the
inclusion complexation of DM-β-CD with ThH. Figure 8 shows a spatial contour plot of the NOESY spectrum for the ThH/DM-β-CD system. Several intermolecular cross-peaks between a-H, b-H, c-H of ThH and 3-H, 5-H of DM-β-CD were observed, indicating that ThH inserted into the hydrophobic cavity of cyclodextrin and the thiophene ring of ThH was deeply included in the CD cavity.

**In Vitro Mucosa Penetration Test** After applying the drug to the mucosa, it must remain physicochemically and enzymatically stable for a certain period of time. All experiments were performed for 2.5 h because it was found that prolonging the experiments over this time would cause the cells could die and the data to become inaccurate. The permeation profiles of ThH and the complexes are illustrated in Fig. 9, expressed as the cumulative amount of ThH permeated per unit surface area (cm²) over time. This showed a linear trend (ThH: $y = 0.9872x -16.6$, $R^2 = 0.9777$, lyophilized complex: $y = 2.4436x -35.768$, $R^2 = 0.9896$). The flux values ($J_s$) of ThH and complex equal to the slope of the linear portion of the plot, which were 0.9872 µg·cm⁻²·min⁻¹ and 2.4436 µg·cm⁻²·min⁻¹, respectively. The $K_p$ values were calculated via the Eq. 4: the mean values of the drug and the complexes were $2.468 \times 10^{-3}$ and $6.109 \times 10^{-3}$ cm·min⁻¹.

The complex crosses the mucosa more easily than its counterpart lacking inclusion. This corresponds to the conclusion reached in the partition coefficient test, in which increases in aqueous solubility and decreases in lipophilicity caused better paracellular transfer of ThH on through the sublingual mucosa. Beyond that, CD is an effective absorption enhancer in mucosal drug delivery. Babu and Pandit have reported that methylated β-CD can extract all the major lipid classes and proteins and reduce the barrier function of the skin.

<table>
<thead>
<tr>
<th>Proton</th>
<th>ThH (δ₀/ppm)</th>
<th>ThH-DM-β-CD complex (δ/ppm)</th>
<th>Δδ (δ₀−δ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-H</td>
<td>7.19</td>
<td>7.16</td>
<td>−0.03</td>
</tr>
<tr>
<td>b-H</td>
<td>6.93</td>
<td>6.85</td>
<td>−0.08</td>
</tr>
<tr>
<td>c-H</td>
<td>6.87</td>
<td>6.74</td>
<td>−0.13</td>
</tr>
<tr>
<td>d-H</td>
<td>6.76</td>
<td>6.74</td>
<td>−0.02</td>
</tr>
<tr>
<td>3-H</td>
<td>3.83</td>
<td>3.87</td>
<td>0.04</td>
</tr>
<tr>
<td>5-H</td>
<td>3.69</td>
<td>3.76</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Fig. 8. Partial Contour Plot of a NOESY Spectrum of ThH-DM-β-CD Complex
Even though the specific mechanism of the action of CDs in increasing the transcellular route has not yet been clarified, it seems likely that the regular arrangement of the lipid molecules that constitute the cell membrane is perturbed by the interaction of membrane lipids and CDs. In addition, interactions between DM-β-CD and biomembranes cannot be excluded; CDs have been shown to interact with membrane lipids and improve permeation and absorption. However, the absorption of group I was very poor. This may have been caused by the high pH in the intestinal tract and the poor dissolution of the DM-β-CD complex. All studies suggested that the good absorption observed after sublingual administration was not caused by swallowing.

**Conclusion**

Formulation with DM-β-CD markedly improved the aqueous solubility of hydrophobic compound ThH and decreased its lipophilicity. The interactions between the drug and excipient were characterized by studies including phase solubility, DSC, X-ray diffractometry, and NMR investigation, in addition, it seems reasonable to postulate that the thiophene ring is deeply included inside the CD cavity. All studies suggested that the preparation of inclusion complex was successful. DM-β-CD was also found to be an effective promoter of permeation through the sublingual mucosa, as indicated by an in vitro mucosa permeation test. Pharmacokinetical profiles and parameters in rats indicated that the inclusion complex was absorbed faster and to a greater overall degree. It was metabolized more slowly when delivered by the sublingual route than through the GIT. The present study demonstrates that the ThH-DM-β-CD method of preparing the inclusion complex and our sublingual delivery system selection are suitable for the pharmaceutical development of ThH.

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