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Characterization and In Vivo Evaluation of an Inclusion Complex of Oridonin and 2-hydroxypropyl-β-cyclodextrin

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Oridonin, a diterpenoid, is a sparingly soluble compound and its aqueous solubility can't meet the requirement of clinical intravenous administration. This study was, accordingly, to prepare an inclusion complex of oridonin and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) by lyophilization to improve its apparent solubility. The solubility enhancement of oridonin was evaluated by phase solubility method, and the phase solubility curve displayed a typical A_L-type, indicating the formation of 1:1 inclusion complex. The formation of inclusion complex was confirmed by DSC, XRD, FTIR, and NMR, and thereby two possible inclusion modes were inferred. In vivo studies demonstrated that HP-β-CD had no significant effect on the plasma pharmacokinetic behaviors of oridonin following i.v. administration to rats, but the inclusion complex tended to decrease the distribution of oridonin in heart, spleen, and kidney and increase that in lung in mice, compared to that of free drug.

Keywords oridonin; 2-hydroxypropyl-β-cyclodextrin; inclusion complex; characterization; pharmacokinetics; tissue distribution

INTRODUCTION

Oridonin (Figure 1A), a diterpenoid isolated from the well-known Chinese herb *Rabdosia rubescens*, has various pharmacological properties such as anti-inflammation, anti-bacteria, and anti-tumor effects (Fuji et al., 1989; Osawa et al., 1994; C. L. Zhang et al., 2003) as well as scavenging active oxygen free radicals (Y. Zhang et al., 1999). It has been demonstrated that oridonin exhibited remarkable inhibitory effects on prostate, breast, non-small cell lung cancers, acute promyelocytic leukemia, and glioblastoma multiforme (Ikezoe et al., 2003; Marks et al., 2002). Its mechanism of action included the reduction of Bcl-2 gene expression and the increase of p53 expression, which results in cycle arrest and apoptosis in LNCaP and NCL-H520 cells (Ikezoe et al., 2003; Marks et al., 2002; C. L. Zhang et al., 2004). However, the clinical application of oridonin has been seriously hindered by its poor aqueous solubility and low therapeutic index (Xing, Zhang, & Tan, 2007). Therefore, it is essential to find an appropriate solution to improve its apparent solubility, thereby facilitating its clinical application.

Cyclodextrins, CDs, are macrocyclic oligosugars most commonly composed of 6, 7, or 8 glucosidic units bearing the names α, β, and γ-CD, respectively (Saenger, 1980). They can include guest molecules of appropriate polarity and dimension due to their hydrophobic central cavity and hydrophilic exterior surface, which makes them widely used in many fields (Beker et al., 1991; Connors, 1997). It has been found that CDs can be used to enhance the solubility and bioavailability of poorly soluble drugs (Dollo et al., 1998; Loftsson & Brewster, 1996; Zingone & Rubessa, 2005), to improve physical and chemical stability of labile drugs (Beker et al., 1991), and to eliminate the undesirable properties of drugs, such as unpleasant odour and taste (Loftsson & Brewster, 1996). Among these CDs, β-CD (Figure 1B) and its chemically modified amorphous 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) (Figure 1B) are the first choices because of their suitable cavity sizes and low prices, and the latter is the most accepted representative of hydroxyalkylated derivatives of β-CD (Beker et al., 1991). HP-β-CD has demonstrated improved complexing ability,

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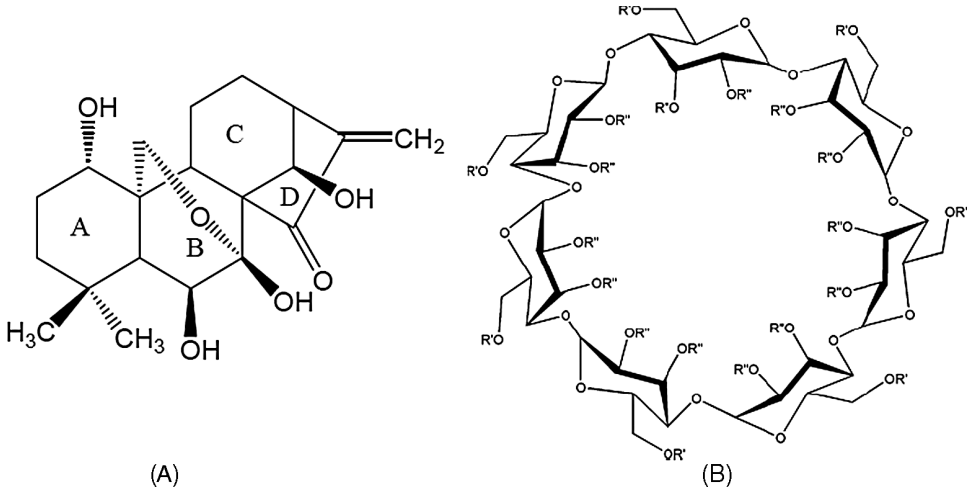

FIGURE 1. Structures of (A) oridonin and (B) β-CD (R′, R″ = H); HP-β-CD (R′, R″ = $CH_2CHOHCH_3$).

greater water solubility, lower toxicity by the parenteral route, and no adverse effects in humans (Carpenter, Gerloczy, & Pitha, 1995). In addition, HP-β-CD has been demonstrated to have little effect on the plasma pharmacokinetics of many drugs (Rajewski & Stella, 1996). However, there are few studies concerning the influence of HP-β-CD on drug distribution to tissues.

Therefore, our interest herein focused on improving the solubility of oridonin by complexation with HP-β-CD. The solubility enhancement of oridonin by inclusion complexation with HP-β-CD was evaluated by phase solubility method. The formation of such a complex was confirmed by DSC, XRD, FTIR, and NMR. Comparison between oridonin free drug and oridonin/HP-β-CD complex solution regarding pharmacokinetics in rats and tissue distributions in mice was carried out; the mechanism of influence of HP-β-CD on oridonin pharmacokinetic behaviors and distribution to tissues is discussed.

MATERIALS AND METHODS

Materials

HP-β-CD (degree of substitution: 0.45) was obtained from Shijiazhuang Pharmaceutical Group (Shijiazhuang, China). Oridonin was obtained from Shannxi Linjing Technology Trade Co., Ltd. (Xi'an, China). Methanol was of high performance liquid chromatography (HPLC) grade. All other reagents and solvents were of analytical grade. Distilled water was used all through the experiment.

Preparation of Inclusion Complex of Oridonin and HP-β-CD

An inclusion complex of oridonin and HP-β-CD was prepared by lyophilization. Oridonin and HP-β-CD were weighted precisely in the 1:1 molar ratio on the basis of the results obtained from the pilot phase solubility studies. Oridonin was dispersed in the aqueous solution of HP-β-CD. The solution was magnetically stirred for 2 days until oridonin was dissolved. The resulting solution was filtered through a 0.22 μm PTFE filter (Shanghai Xinya Cleaning Device Co., China). The filtrate was frozen and then lyophilized.

Preparation of Physical Mixture of Oridonin and HP-β-CD

A physical mixture consisting of oridonin and HP-β-CD in the 1:1 molar ratio was prepared. The oridonin and HP-β-CD were added in a mortar and mixed for 10 min to obtain a homogeneous blend.

Phase Solubility Studies

The solubility method was employed according to the method of Higuchi and Connors (1965). The excess amount of oridonin (75 mg) was added in the screw capped vials containing HP-β-CD solutions (3 ml) at various concentrations (0 –164 mM). The vials were shaken at 25°C. After equilibrium was attained (2 days), the samples were prepared by filtering through a 0.22 μm PTFE filter, and analyzed by high-performance liquid chromatography (HPLC). Appropriate dilutions were made with methanol such that the final concentration was within the linear portion of the standard curve for oridonin prior to injection into the HPLC column. All samples were prepared in triplicate.

Oridonin was analyzed by HPLC (Yuan et al., 2004) with the following conditions: a Hitachi Instruments HPLC System (Hitachi, Japan), L7110 pump and L7420 UV detector at 242 nm; Kromasil C18 reversed-phase column 150 × 4.6 mm (5 μm); a mobile phase of methanol: water (50:50 v/v); a flow rate of 1.0 ml/min and a column temperature of 40°C. In these conditions, oridonin was eluted as a well-defined peak without any interference from samples. The peak area (A) vs. concentration (C)

was linear over the range of 0.5–10 μg/ml, fitting the equation: A = 27247 C – 1577.5 (r = 0.9999). At concentrations of 2.0, 5.0, and 8.0 μg/ml, recovery of oridonin from HP-β-CD solution was 101.0%, 98.87%, and 101.2%, RSD of oridonin peak areas were 1.20%, 1.00%, and 0.98%, respectively. It was validated that there was no effect of HP-β-CD on the retention time and peak area of oridonin in aqueous solution.

Differential Scanning Calorimetry (DSC) Analysis

DSC measurements were performed by a DSC-60 differential scanning calorimeter (Shimadzu, Japan). The weighed samples were placed in aluminum pans and empty aluminum pans were used as references. The experiments were carried out under nitrogen flow (20 ml/min) at a scanning rate of 10°C /min in the range of 20 – 350°C.

Powder X-Ray Diffractometry (XRD)

Powder X-ray diffraction patterns were obtained from a Ricoh Dmax2400 diffractometer (Ricoh, Japan) over 2 θ angles of 3° to 40°, with an electric current of 150 mA and a voltage of 50 kV, at 25°C.

Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectra were obtained using a Bruker IFS-55 spectrometer (Bruker, Switzerland). The samples were previously ground and mixed thoroughly with KBr; The KBr disks were prepared by compressing the powder. The scans were executed from 4000 to 400 cm^{-1}spectral region.

Nuclear Magnetic Resonance (NMR) Study

The two-dimensional rotating frame Overhauser effect (ROESY) spectra were obtained on a Bruker AC600 DRX NMR spectrometer (Bruker, Switzerland), measured under the following conditions: relaxation delay = 1 s, mixing times = 300 ms, number of scans = 16. The inclusion complex of oridonin and HP-β-CD were dissolved in D_2O.

Pharmacokinetics in Rats and Tissue Distribution in Mice

Pharmacokinetics studies were performed using male Wistar rats weighing approximately 230–250 g, obtained from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The animal experimentation was approved by the Animal Ethics Committee of Shenyang Pharmaceutical University (Shenyang, China). A group of six rats was injected the solution of inclusion complex at a dose of 10 mg oridonin/kg rats in the femoral vein. Blood samples (0.25 ml) were collected in heparinized tubes from each rat at 0.083, 0.167, 0.5, 1, 2, 4, 6, 9, 12, 24, 36, and 48 h after administration, and were immediately centrifuged and stored at –20°C until analysis.

Tissue distribution studies were performed using male Kunming mice weighing approximately 22–25 g, obtained from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The mice were randomly divided into two groups. The reference group was injected oridonin (5 mg/ml) prepared with a 30% alcohol aqueous solution at a dose of 20 oridonin mg/kg mice in the caudal vein, and the test group was injected the solution of inclusion complex at the same dose. Five mice in each group were sacrificed at 0.083, 1.0, and 6.0 h after administration, respectively, and heart, liver, spleen, lung, kidney, and brain were dissected. The tissues were washed with normal saline, homogenized, and stored at –20°C until analysis. Oridonin in plasma and tissues was quantified by an HPLC/ESI-MS method developed by our laboratory (Xu et al., 2006a).

The area under the plasma concentration–time curve from time zero to infinity ($AUC_{0-\infty}$) was calculated from the equation $AUC_{0-\infty} = AUC_{0-t} + C_t/k$, where AUC_{0-t} was calculated using the linear trapezoidal method; C_t is the last quantifiable concentration and k was calculated from the slope of a straight-line in the terminal phase of plasma disappearance. The terminal phase half-life ($t_{1/2}$) was calculated as: $t_{1/2} = 0.693/k$. The following parameters also were calculated: the total plasma clearance (CL) = Dose/$AUC_{0-\infty}$; the steady-state volume of distribution (Vss) = CL•MRT; and the mean residence time (MRT) = AUMC/AUC, where AUMC represents the area under the moment curve.

RESULTS AND DISCUSSION

Phase Solubility

The phase solubility technique is very useful for investigating inclusion complexation of poorly water-soluble drugs with CDs in water, because it gives not only the solubilizing ability of CDs but also the stability constant of the complexes by analyzing the solubility curve (Higuchi & Kristiansen, 1970). Figure 2 shows the phase solubility diagram of oridonin and HP-β-CD. It was observed that the apparent solubility of oridonin showed a linear relationship with HP-β-CD

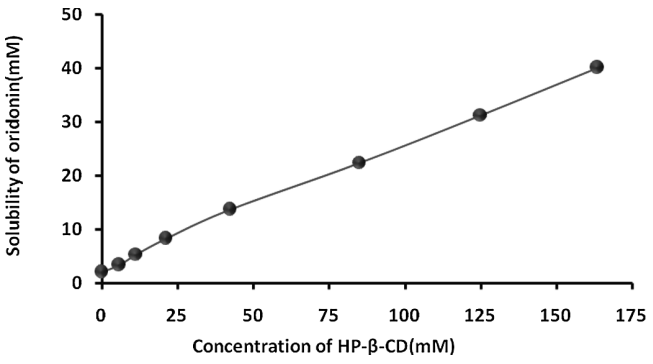

FIGURE 2. Phase solubility diagram of oridonin and HP-β-CD at 25°C.

concentration (r = 0.9989). The phase solubility curve displayed a typical A_L- type, indicating the formation of 1:1 inclusion complex during the HP-β-CD concentration range investigated.

The inclusion stability constant $K_{1:1}$ was estimated to be 107.7 ± 6.9 M^{-1} from the slope (0.2297) and intercept S_0 (2.7677 mM) of the linear phase solubility curve, according to the following equation:

$$K_{1:1} = \frac{\text{Slope}}{S_0(1-\text{Slope})} \quad (1)$$

The highest solubility of oridonin in the presence of HP-β-CD also was investigated and reached 55.45 ± 0.09 mM when using 260 mM HP-β-CD, demonstrating a nearly 27-fold increase of oridonin solubility compared to the equilibrium solubility of oridonin (2.062 mM, 25°C).

For drugs with intrinsic solubility < 1 mg/ml, it is possible that the inclusion stability constant $K_{1:1}$ obtained from the phase solubility method isn't reliable due to the inaccurate determination of S_0 from the intercept of the linear phase solubility curve (Loftsson, Hreinsdottir, & Masson, 2005). So the complexation efficiency (CE), which means the ratio of the complex to free cyclodextrin concentration, was calculated according to the following equation (Loftsson, Masson, & Sigurjonsdottir, 1999):

$$CE = S_0 \cdot K_{1:1} = \frac{[D/CD]}{CD} = \frac{\text{Slope}}{1-\text{Slope}} \quad (2)$$

Where [D/CD] is the concentration of dissolved complex, [CD] is the concentration of dissolved free HP-β-CD and slope is the slope of the phase solubility curve. The CE was calculated to be 29.8%, suggesting that on an average about one out of every four HP-β-CD molecules in solution took part in forming a water-soluble inclusion complex with oridonin (Loftsson et al., 2005).

DSC Analysis

DSC can be used for characterizing the inclusion complexes. When guest molecules are included in CD cavities or crystal lattice, their melting, boiling, and sublimation points shift to different temperatures or disappear. The thermograms of oridonin, HP-β-CD, physical mixture, and inclusion complex are shown in Figure 3. The DSC diagram of oridonin exhibited a sharp endothermic peak at 271°C, indicating the melting point of oridonin. The curve of HP-β-CD showed a broad endothermic effect between 280°C and 340°C, which

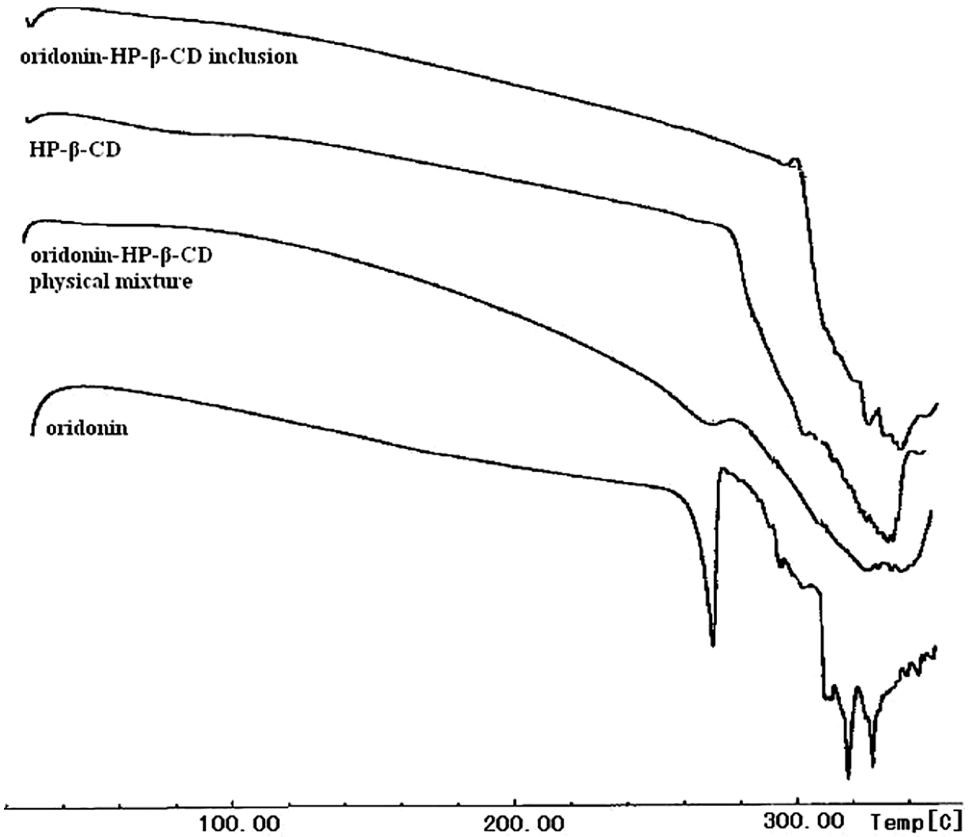

FIGURE 3. DSC thermograms of oridonin, HP-β-CD, physical mixture, and inclusion complex.

attained a maximum around 330°C, equivalent to a degradation temperature of HP-β-CD. For the physical mixture of oridonin and HP-β-CD, there also was a broad peak between 280°C and 340°C, and a smaller peak around 271°C, which was likely to be the melting point of oridonin. Since the thermogram of the physical mixture was similar to the superimposition of the thermograms of individual oridonin and HP-β-CD, there might be absent of interaction between oridonin and HP-β-CD in the physical mixture (Marques, Hadgraft, & Kellaway, 1990). The thermogram of the inclusion complex did not show any sharp endothermic peak in the temperature range investigated, which indicated the amorphous character of this sample and the existence of interaction between oridonin and HP-β-CD. The disappearance of the melting peak of oridonin from the thermogram of the inclusion complex might be due to the crystalline oridonin being included within the central cavity of the HP-β-CD ring molecule.

Powder XRD

The formation of inclusion complex also was identified by X-ray powder diffraction as demonstrated in Figure 4. The X-ray powder diffractogram of oridonin exhibited an intense sharp peak, displaying its crystalline character. Pattern of HP-β-CD indicated an amorphous structure by the lack of intense sharp peaks. The pattern of the physical mixture of oridonin and HP-β-CD showed an intense sharp peak similar to that of the pure oridonin sample. It could be observed that the pattern of the physical mixture was likely to the superimposition of the patterns generated by the each component, indicating that there was no formation of a new crystal form. However, compared to the diffractogram of oridonin and the physical mixture, the sharp peak completely disappeared as can be seen from the pattern of the inclusion complex, indicating the amorphous nature of the structure. This lack of crystallinity provided an evidence for the formation of the inclusion complex.

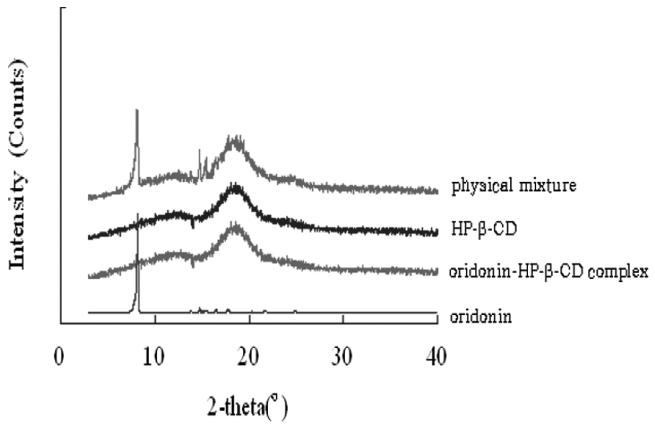

FIGURE 4. X-ray diffractograms of oridonin, HP-β-CD, physical mixture, and inclusion complex.

FTIR Spectroscopy

Further analysis of inclusion complex was obtained by FTIR spectroscopy. The FTIR spectra of wave number from 4000 to 400 cm^{-1} are presented in Figure 5. The carbonyl has an intense absorption band between 1680 and 1750 for its stretching vibration, which is the characteristic peak for identification of carbonyl. When carbonyl is conjugated with double bond, its stretching vibration should shift to low wave number region. As can be seen from Figure 5, oridonin was characterized by the intense absorption peak at 1710 cm^{-1} for carbonyl stretching vibration, whereas lack of absorption peak at the same wave number was observed in the spectrum of HP-β-CD.

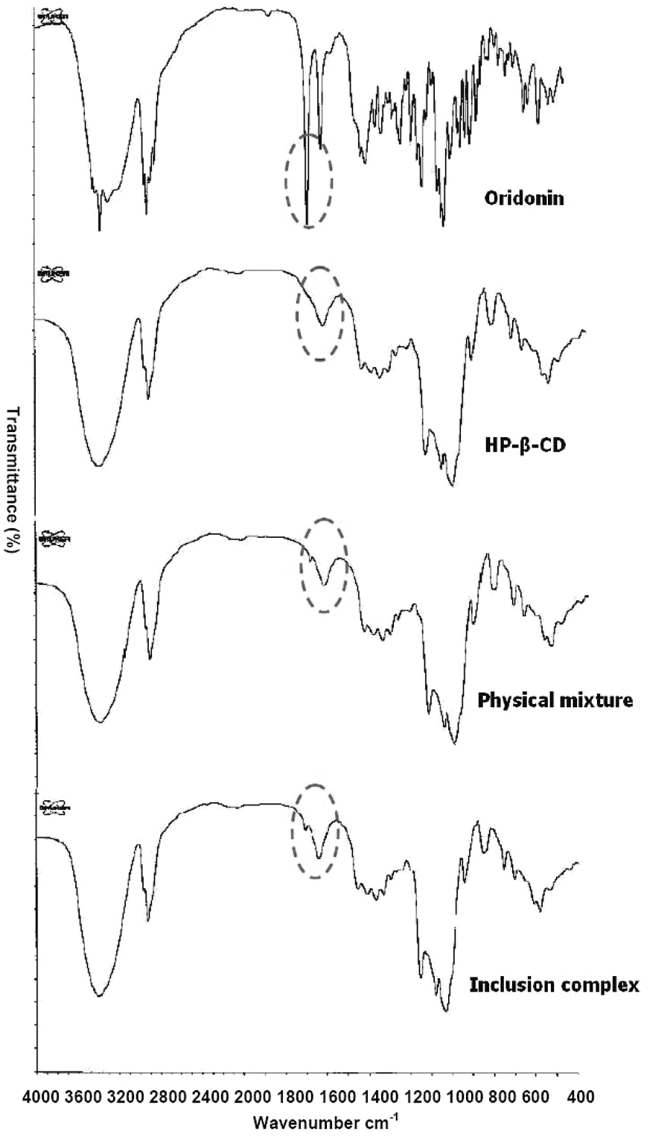

FIGURE 5. FTIR spectra of oridonin, HP-β-CD, physical mixture, and inclusion complex.

In the physical mixture of oridonin and HP-β-CD, the spectrum showed a less intense absorption peak at 1710 cm^{-1}, and no other obvious characteristic peaks of oridonin were observed due to the low ratio of oridonin in mixture. What is notable was that the spectrum of inclusion complex was extremely similar to that of physical mixture, also showing a less intense absorption peak at 1710 cm^{-1}. This indicated that there was no interaction between the carbonyl of oridonin molecule and the HP-β-CD molecule, and that the carbonyl was not included in the central cavity of HP-β-CD ring molecule. Therefore, it was inferred that only a part of oridonin molecule (ring A, Figure 1A) was included in the cavity, and that the methylene cyclopentanone portion (ring D, Figure 1A) of oridonin molecule was not included.

NMR Study

In order to elucidate the space conformation of the inclusion complex, the ROSEY experiment was carried out to obtain further information. The two-dimensional ROSEY spectrum of the inclusion complex is shown in Figure 6, where peaks of oridonin were identified on the top of the figure and peaks of HP-β-CD were marked on the right. The cross-peaks were observed between the Hc, Hd, He, Hf, Hg, and Hh protons of the drug and the H_3 and H_5 protons of glucosidic units of HP-β-CD, shown by the points of intersection of the three dash lines. So, interaction between the methyls of the drug and the H_3 and H_5 protons was confirmed, indicating that the ring A of oridonin molecule inserted into the HP-β-CD torus. In contrast, a lack of cross-peaks was observed between the Ha, Hb

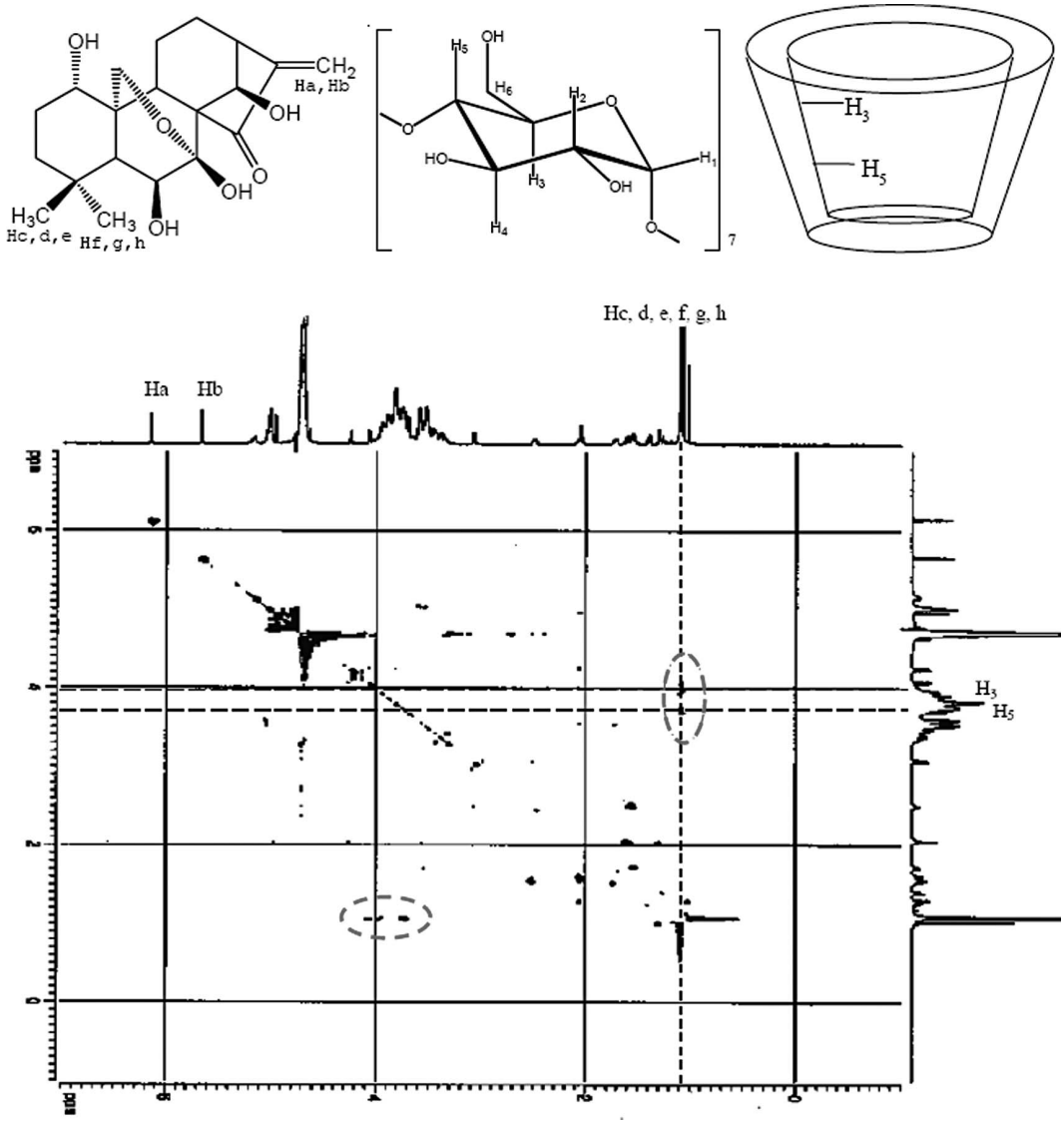

FIGURE 6. Partial contour plot of ROSEY spectrum of inclusion complex of oridonin and HP-β-CD.

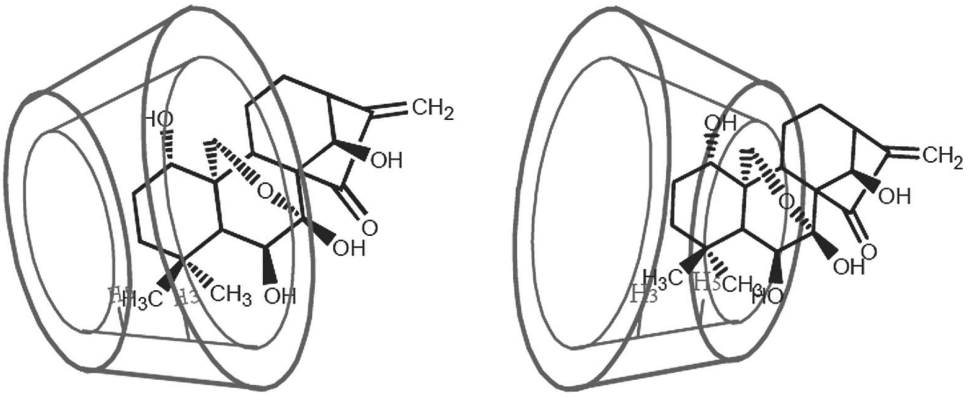

FIGURE 7. Two possible inclusion modes of inclusion complex of oridonin and HP-β-CD.

protons of the drug and the H_3, H_5 protons of HP-β-CD, which indicated that the ring D of the drug molecule was not included in the cavity of HP-β-CD molecule, in line with the results of FTIR spectra. Since both H_3 and H_5 protons of HP-β-CD had interaction with the methyls of oridonin, as shown in Figure 6, the ring A of oridonin molecule could insert into the HP-β-CD torus either from the wide rim or the narrow one. The two possible inclusion modes are shown in Figure 7.

Pharmacokinetics in Rats and Tissue Distribution in Mice

The mean concentration-time curves in plasma following i.v. administration to rats are shown in Figure 8, in which the data for the oridonin in cosolvent solution, studied by our laboratory (Xu et al., 2006b), also are included for comparison. The result demonstrated that oridonin was quickly distributed into tissues, followed by a stable phase before a slow elimination. There was little effect of HP-β-CD on the pharmacokinetic behavior of oridonin, except for a slightly more rapid distribution. The non-compartmental pharmacokinetic parameters obtained are listed in Table 1, including the data for the oridonin free drug (Xu et al., 2006b) and the inclusion complex. No statistical differences were observed in AUC valueand other pharmacokinetic parameters between them ($P < 0.05$). These results are consistent with the earlier studies on the inclusion complexes showing that CD derivatives had no effects on the i.v. plasma pharmacokinetics of the drugs investigated (Kim et al., 2004; Liu, Qiu, Gao, & Jin, 2006; Piel, Evrard, Van, & Delattre, 1999; Zuo, Tam, Diakur, & Wiebe, 2002).

The similar plasma pharmacokinetic behaviors between the two groups may be ascribed to the rapid release of large fraction of oridonin from HP-β-CD because of the effect of dilution, binding of drug to a plasma protein and binding of a competing agent to the HP-β-CD (Rajewski & Stella, 1996; Stella, Rao, Zannou, & Zia, 1999). Since the process of releasing drug from the complex can be completed rapidly (Hahimoto & Thomas, 1985; Turro, Okubo, & Chung, 1982), the complex of oridonin and HP-β-CD behaved approximately as a true solution in the plasma. Although pharmacokinetic behaviors of the two dosage forms were similar, the inclusion complex was obviously more preferable than the cosolvent solution since injection of the inclusion complex would not result in drug precipitation or local irritation,

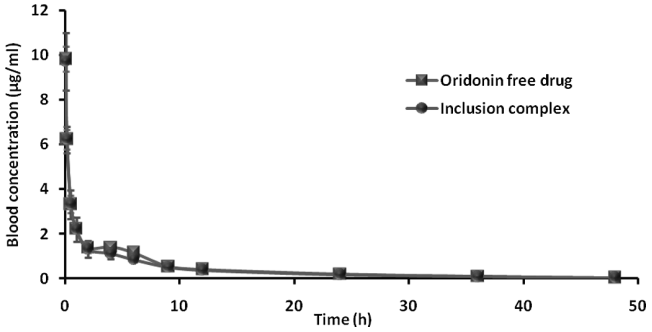

FIGURE 8. Mean concentration-time curves of the oridonin in plasma. Either the oridonin free drug (Xu et al., 2006b) or the inclusion complex was intravenously administrated to rats at a dose of 10 mg oridonin/kg rats. Each point represents the mean ± *SD* of six rats.

TABLE 1
Non-Compartmental Pharmacokinetic Parameters of Oridonin Following I.V. Administration of the Oridonin Free Drug (Xu et al., 2006b) and Inclusion Complex at a Dose of 10 mg /kg Rats (Mean ± *SD*, $n = 6$)

Parameter	Reference Group	Test Group
Ke (h^{-1})	0.0634 ± 0.0025	0.0630 ± 0.0045
$t_{1/2}$ (h)	10.94 ± 0.43	11.04 ± 0.86
$AUC_{0-\infty}$ (mg•h/L)	22.55 ± 1.77	19.66 ± 5.12
MRT(h)	10.36 ± 0.69	10.79 ± 0.98
CL(L/h/kg)	0.45 ± 0.04	0.54 ± 0.14
Vss(L/kg)	4.89 ± 0.42	5.86 ± 1.86

which might occur with the cosolvent solution (Rajewski & Stella, 1996).

The results of tissue distribution after i.v. administration to mice are presented in Figure 9. The tissue distribution of oridonin for the inclusion complex showed some differences from that of the free drug. After i.v. administration of the oridonin/HP-β-CD complex, we could observe lower concentration in heart, kidney, and spleen, but higher concentration of oridonin in lung than that of the free drug. This finding suggested that HP-β-CD could, to some extent, alter the tissue distribution of oridonin in mice.

The lower drug concentration in heart, kidney, and spleen with inclusion complex can be explained by the equilibrium mechanism of the drug after i.v. administration (Figure 10). Figure 10(A) shows the process of binding oridonin to a plasma protein after administration by cosolvent solution, and Figure 10(B) shows three equilibria processes after administration by inclusion complex: binding oridonin to a HP-β-CD, binding oridonin to a plasma protein, and binding a competing agent to a HP-β-CD (Stella et al., 1999). K, K_P, and K_C are the binding constants for the drug/HP-β-CD complexation, drug/protein complexation, and competing agents/HP-β-CD complexation, respectively. Each of these binding constants can be defined by Equations 3, 4, and 5.

$$K = \frac{[ORI/CD]}{[ORI][CD]} \tag{3}$$

$$K_P = \frac{[ORI/P]}{[ORI][P]} \tag{4}$$

$$K_C = \frac{[C/CD]}{[C][CD]} \tag{5}$$

From Figure 9, we know that compared with cosolvent solution, the complexation of oridonin and HP-β-CD can decrease the concentration of free oridonin in the plasma and, thereby, decrease the distribution of oridonin to heart, kidney, and spleen, since the inclusion complex is unable to penetrate through cell membranes, although it is able to pass the

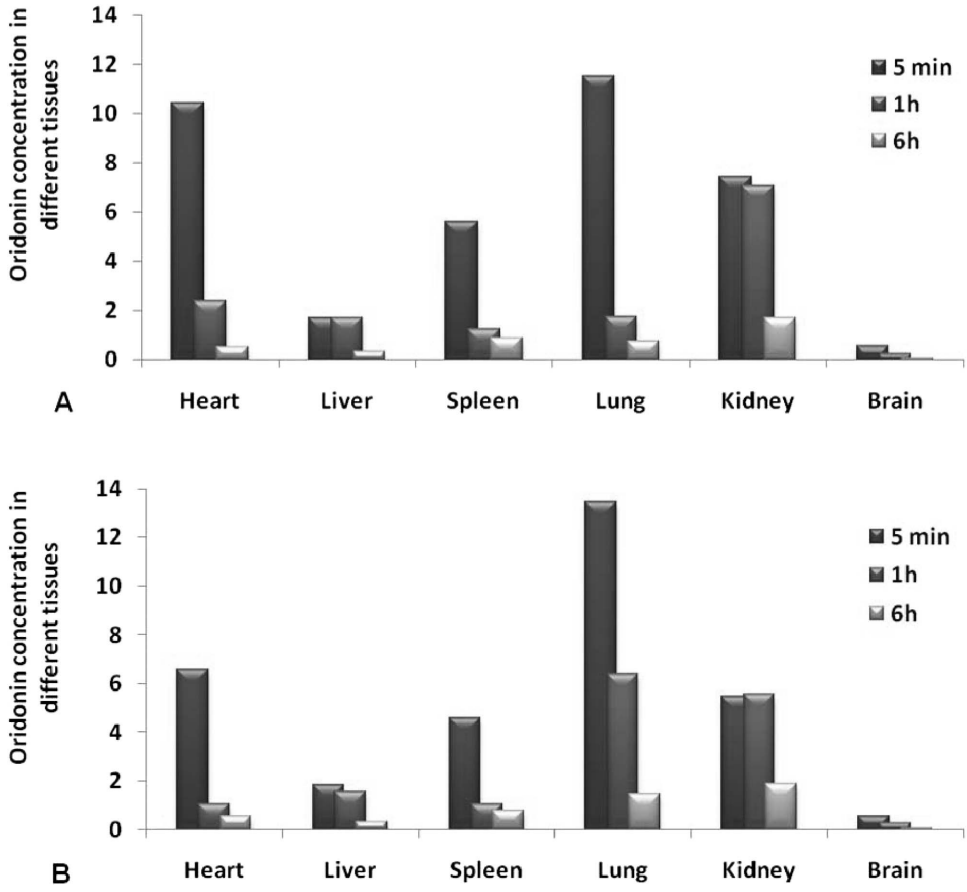

FIGURE 9. Tissue distribution of oridonin in mice following i.v. administration of (A) oridonin free drug and (B) oridonin/HP-β-CD complex at a dose of 20 mg oridonin/kg mice (n = 5).

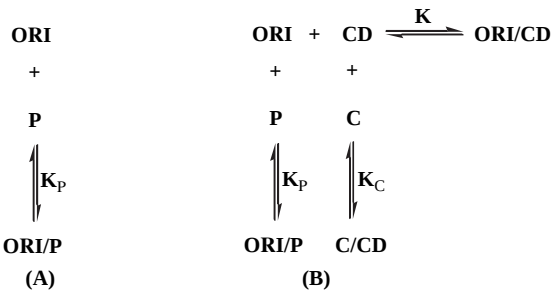

FIGURE 10. Equilibrium mechanism of oridonin after i.v. administration for (A) co-solvent solution and (B) inclusion complex.

vascular endothelium (Frijlink et al., 1990). But the differences between the two dosage forms indicated from the results are not very striking, which can be explained by the fact that the K of oridonin is relatively small (K = 107 M^{-1}), and that the complexation between competing agents and HP-β-CD can further weaken the effect of HP-β-CD on decreasing the concentration of free oridonin.

The results in this study differed from those reported by Frijlink (Frijlink et al., 1991), concerning the effects of HP-β-CD on the distribution of flurbiprofen. The distributions of flurbiprofen in liver, brain, kidney, and spleen were increased by HP-β-CD, whereas in the present study the oridonin distributions in these tissues were decreased by HP-β-CD. The differences might be due to different K and K_P for the two drugs. Flurbiprofen has relatively high K (K=12515 M^{-1}) and K_P, determining that most drug molecules in the plasma are, not in free state, but binding to proteins after administration of cosolvent solution, or binding to either proteins or HP-β-CD after administration of inclusion complex. Therefore, the difference of flurbiprofen distribution between the two dosage forms is determined more by whether it is binding to proteins or to HP-β-CD, less by the difference of free flurbiprofen concentrations in plasma between the two dosage forms. Frijlink et al., 1991, ascribed the difference of flurbiprofen distribution to an inference that HP-β-CD is able to deliver flurbiprofen to biological membranes in a more efficient way than the plasma proteins. In contrast, oridonin has a relatively low K_P, which can be inferred, to some extent, from the rapid decrease of oridonin concentration in the plasma following i.v. administration to rats, and a low K. Accordingly, oridonin molecules are mainly in free state in plasma following administration of either cosolvent solution or inclusion complex, and the difference of oridonin distribution between the two dosage forms is determined more by the difference of free oridonin concentrations in plasma between the two dosage forms, less by whether it is binding to proteins or to HP-β-CD. Consequently, since the free oridonin concentration in plasma can be decreased by complexation of oridonin and HP-β-CD, the distributions of oridonin to these tissues can be also decreased.

The increased oridonin distribution in lung by HP-β-CD might be explained by an inference that HP-β-CD has a relatively high concentration in lung due to its possible binding to some specific substances in lung, which results in a higher oridonin distribution to lung. Further studies should be carried out to verify the inference.

Another interesting result was that the oridonin concentration in kidney at 1 h is higher than that of 5 min, which was neither observed in reference group, nor in other tissues. This result may be due to the retaining effect of HP-β-CD on the lipophilic drugs in kidney proposed by Stella (Stella et al., 1999). After a drug is cleared by filtration in kidney, water reabsorption in the proximal and distal tubules results in about a 100-fold increase in the concentration of the drug. During this step, lipophilic drugs, like oridonin, normally undergo passive reabsorption, while hydrophilic agents like HP-β-CD are simply concentrated. The increased concentration of HP-β-CD encourages the complexation of oridonin and HP-β-CD, thereby inhibits passive reabsorption of oridonin, and retains oridonin at a relatively high concentration in the renal tubule for a long time.

In conclusion, an inclusion complex of oridonin and HP-β-CD was successfully prepared by lyophilization method and characterized by DSC, XDR, FTIR, and NMR. The apparent solubility of oridonin was greatly enhanced by forming inclusion complex with HP-β-CD. In vivo studies demonstrated that HP-β-CD had no significant effect on the i.v. pharmacokinetics of oridonin in rats, while it changed the tissue distributions in mice to some extent. The results of this paper provide an effective method to improve the apparent solubility of oridonin, and an important reference for the clinical application of oridonin.

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