

## Complexation of Z-ligustilide with hydroxypropyl- $\beta$ -cyclodextrin to improve stability and oral bioavailability

YAPENG LU<sup>1</sup>  
SIYUAN LIU<sup>2</sup>  
YU ZHAO<sup>1</sup>  
LI ZHU<sup>1,\*</sup>  
SHUQIN YU<sup>3,\*\*</sup>

<sup>1</sup> Institute of Nautical Medicine, Nantong University, Nantong 226001, China

<sup>2</sup> School of Life Sciences, Nantong University, Nantong 226007, China

<sup>3</sup> Jiangsu Key Laboratory for Supramolecular Medicinal Materials and Applications College of Life Sciences, Nanjing Normal University, Nanjing 210046, China

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To improve the stability and oral bioavailability of Z-ligustilide (LIG), the inclusion complex of LIG with hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) was prepared by the kneading method and characterized by UV-Vis spectroscopy, differential thermal analysis (DTA) and Fourier transform infrared (FTIR) spectroscopy. LIG is capable of forming an inclusion complex with HP- $\beta$ -CD and the stoichiometry of the complex was 1:1. Stability of the inclusion complex against temperature and light was greatly enhanced compared to that of free LIG. Further, oral bioavailability of LIG and the inclusion complex in rats were studied and the plasma drug concentration-time curves fitted well with the non-compartment model to estimate the absolute bioavailability, which was 7.5 and 35.9 %, respectively. In conclusion, these results show that LIG/HP- $\beta$ -CD complexation can be of great use for increasing the stability and biological efficacy of LIG.

**Keywords:** Z-ligustilide, hydroxypropyl- $\beta$ -cyclodextrin, inclusion complex, stability, bioavailability

Radix *Angelica sinensis*, known as Danggui and mainly spread in Gansu and Sichuan provinces of China, has been used for a long time as a traditional Chinese medicine to treat cardiovascular and cerebrovascular diseases (1, 2) as well as a common food supplement to reinforce vital energy (3, 4). Modern phytochemical studies have shown that LIG (Fig. 1) is the main lipophilic component of Danggui (1). Our previous studies have shown that LIG had neuroprotective effects on some models of diseases associated with cerebral ischemia. Treatment with LIG could significantly improve behavioral deficits and dose-dependently reduce cerebral infarct volumes after focal cerebral ischemia in rats (1, 5). It could also prevent chronically hypoperfused cognitive deficits and brain damage in rats induced by permanent ligation of both common carotid arteries (6). A recent study has shown that LIG has a neuroprotective effect on Alzheimer's disease, cognitive impairment and neuropathological changes induced by bilateral intracerebroven-

\* Correspondence; e-mail: zhuli85051796@gmail.com; yushuqin@njnu.edu.cn; Yapeng Lu and Siyuan Liu contributed equally to this work.

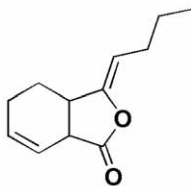


Fig. 1. Z-ligustilide structure.

tricular injections of  $A\beta_{25-35}$  (7). These findings suggest that LIG has the potential to be developed into a therapeutic agent for preventing and treating ischemic disorders and Alzheimer's disease. However, the poor solubility and stability of LIG have strongly influenced its oral formulation development (8). Therefore, considerable attention has been paid to increasing the oral bioavailability and physicochemical stability of LIG and developing its high-quality oral formulation.

In clinical practice, there is a strong argument that oral treatment is essential for chronic diseases of the central nervous system. Oral delivery also improves patients' compliance and comfort as well as development of chronic treatment schedules, which would decrease the cost of therapy. Extensive efforts are being focused on resolving the issue of poor bioavailability of clinically important drugs by employing various pharmaceutical approaches (9).

Cyclodextrins (CDs) are cyclic oligosaccharides made up of six ( $\alpha$ -cyclodextrin), seven ( $\beta$ -cyclodextrin) and eight ( $\gamma$ -cyclodextrin) glucopyranose units linked by  $\alpha$ -(1,4) bonds (10). CDs can interact with appropriately sized molecules to form inclusion complexes. These inclusion complexes have been successfully used to improve the solubility, stability and bioavailability of many compounds. However, the application of CDs is limited by their rather low aqueous solubility (11). HP- $\beta$ -CD is a hydroxyalkyl  $\beta$ -cyclodextrin derivative widely studied in the fields of drug development and food industry because of its inclusion ability as well as its high water solubility (12, 13). In addition, toxicological studies have pointed out that HP- $\beta$ -CD is well tolerated by the human body both in intravenous and oral administration (12).

More recently, several studies have been performed on the inclusion complexation of HP- $\beta$ -CD with natural products such as artemether (14) and astaxanthin (15), but no inclusion complex of LIG with HP- $\beta$ -CD has been reported to date. In this study, we investigated the complexation of LIG with HP- $\beta$ -CD with the aim to improve the bioavailability and stability of this compound. The complex was prepared by the kneading method at the stoichiometric ratio. UV-Vis spectroscopy, DTA and FTIR spectroscopy were used to characterize the complex. In addition, the stability and oral bioavailability of the LIG/HP- $\beta$ -CD inclusion complex in rats were evaluated and compared with that of free LIG.

## EXPERIMENTAL

### Materials

Danggui was purchased from the Danggui Cultivating Base of Good Agricultural Practice in Nin Xian County, Gansu Province, China, and LIG was isolated by silica-gel

column chromatography, which is a well-established procedure in our laboratory (16). Its purity was over 97 % based on HPLC analysis. LIG standard substance (98.5 %) was purchased from J&K Scientific Ltd. (China). HP- $\beta$ -CD (DS = 6.0) was purchased from Taixin Yimin Chemical Co. Ltd. (China). All water was distilled and deionized using a Milli-Q Plus system (Millipore, USA). Acetonitrile (HPLC grade) was purchased from Fisher Scientific (USA). Other reagents and chemicals were of analytical reagent grade.

### *UV-Vis spectroscopic studies*

UV-visible spectroscopy is an important tool to study complexation of guest molecules with CDs by the shift of absorption maximum and absorbance of these molecules in the presence of CDs (17). LIG and HP- $\beta$ -CD were dissolved in a mixed solution of ethanol and water (1:1, V/V). A 1.0 mL portion of  $3 \times 10^{-4}$  mol L<sup>-1</sup> LIG was transferred accurately into a 10 mL standard flask, 1.0 mL  $10^{-2}$  mol L<sup>-1</sup> HP- $\beta$ -CD was added sequentially, diluted to the mark with the same solution and mixed well for 10 min with an ultrasonic generator. The solution was left to stand for 10 min at room temperature. The UV-Vis absorption spectra of the LIG/HP- $\beta$ -CD complex were recorded against a reagent blank prepared with the same reagent concentration but no LIG. In addition, absorption spectra of LIG and HP- $\beta$ -CD were recorded according to the same procedure.

### *Job's plots*

Stoichiometry of the inclusion complex was determined by Job's method of continuous variation (18). Job's plot was determined from UV spectrophotometry data according to the continuous variation method. LIG and HP- $\beta$ -CD were dissolved in a mixed solution of ethanol and water (1:1, V/V). Equimolar  $3 \times 10^{-4}$  mol L<sup>-1</sup> solutions of LIG and HP- $\beta$ -CD were mixed to a standard volume varying the molar ratio but keeping the total concentration of the species constant. To calculate stoichiometry, the absorbance at 328 nm was measured for all solutions and the difference in absorbance of LIG ( $\Delta A$ ) in the presence and absence of HP- $\beta$ -CD was plotted against  $R(R=[LIG]/([LIG]+[HP-\beta-CD]))$ . Spectra were obtained with a Shimadzu UV-2450 spectrophotometer (Shimadzu, Japan). Each complex solution was measured in triplicate.

### *Preparation of the inclusion complex of LIG with HP- $\beta$ -CD*

An inclusion complex of LIG with HP- $\beta$ -CD was prepared by the kneading method (19). LIG and HP- $\beta$ -CD were weighted precisely in the 1:1 molar ratio on the basis of the results obtained from Job's plots studies. Specifically, LIG was dissolved in ethanol. Drops of LIG solution were added to HP- $\beta$ -CD, which was wetted with the same solvent and kneaded thoroughly. During the kneading, drops of ethanol were added to the mixture in order to maintain its consistency as a paste. The obtained solid mixture was then washed sequentially with water and ethanol. Finally, the mixture was frozen at  $-80$  °C and subsequently freeze-dried for 24 h at  $-55$  °C using the Labconco Freeze Dry System (Labconco, USA).

A physical mixture consisting of LIG and HP- $\beta$ -CD in the same 1:1 molar ratio was prepared. LIG and the HP- $\beta$ -CD were added into a mortar and mixed slightly for 5 min

with a small amount of ethanol (minimum amount to form a slurry) to obtain a homogeneous blend. Finally, the physical mixture was frozen and then lyophilized in the same way as the complex was prepared.

### *Differential thermal analysis (DTA)*

DTA curves of LIG, HP- $\beta$ -CD, LIG/HP- $\beta$ -CD inclusion complex and their physical mixture were measured with a DTA-60 differential thermal analyzer (Shimadzu, Japan). Each sample (3–5 mg) was weighed and heated in an aluminum pan at a rate of 10.00 °C min<sup>-1</sup> in a 20 to 400 °C temperature range under constant flow (30 mL min<sup>-1</sup>) of dry nitrogen.

### *FTIR spectroscopy*

FTIR was conducted using a Nicolet 5DXC IR Spectrometer (Nicolet, USA). The diffuse reflectance technique was used in the mid-IR (400–4000 cm<sup>-1</sup>) spectral region. The procedure consisted of grinding the sample together with KBr (about 200–400 mg) into a fine powder, placing the powder into a sampling cup, smoothing it, and compressing the powder bed into the holder using a compression gauge. The sample was placed in the light path and the spectrum was obtained.

### *Stability of the inclusion complex*

LIG and LIG/HP- $\beta$ -CD inclusion complexes were divided into four groups which were stored at 4, 30 and 50 °C (in brown glass bottles) and under light (with light intensity 1500 Lux, 4 °C), respectively. Comparative tests of the stability of free LIG and the inclusion complex were run for 6 days. After a fixed period of time, the remaining LIG in each sample was measured using HPLC analysis. Each test was repeated three times.

### *HPLC analysis of LIG levels from in vitro samples and biological samples*

A HPLC system (Shimadzu, Japan) provided with a Shimadzu CBM 20A controller, two Shimadzu LC 20 AD pumps including a degasser, a Shimadzu CTO 20 AC column oven and a Prominence SPD 20A UV-deuterium detector was employed. Separation (20  $\mu$ L injection volume) was carried out on a Kromasil ODS C<sub>18</sub> column (250 mm  $\times$  4.6 mm, 5  $\mu$ m particle size) and the detection wavelength was 328 nm. The mobile phases used for the analysis consisted of acetonitrile/water (containing 10 % triethylamine) (65:35), adjusted to pH 3.0 with glacial acetic acid, delivered at a flow rate of 1 mL min<sup>-1</sup>.

### *Bioavailability studies*

Male SD rats weighing between 240 g and 260 g were fasted for 12 h before drug administration. All the animals were maintained in the laboratory animal facility (22  $\pm$  2 °C, 50  $\pm$  5 % relative humidity, 12-h light/dark cycle). The rats were acclimated to the laboratory environment for 1 week before starting the experiment. Water was freely available. All pharmacokinetic studies reported herein were submitted to the ethics committee on animal experimentation of Nantong University and all procedures were approved by the Animal Care and Use Committee of Nantong University and the Jiangsu Province Animal Care Ethics Committee (Approval ID: SYXK(SU)2007-0021).

For intravenous (*i.v.*) administration, free LIG was suspended in 0.5 % Tween-80. Intravenous bolus was given to rats *via* the tail vein at a dose of 20 mg kg<sup>-1</sup>. Blood samples were collected and centrifuged at 0.08, 0.25, 1, 1.5, 2, 4, 8 h post-dosing. For the LIG assay, 900  $\mu$ L hexane and 10  $\mu$ L internal standard (Nimodipine, 100  $\mu$ g mL<sup>-1</sup>) were added to the 300  $\mu$ L plasma sample. After centrifugation, the supernatant was withdrawn and the solvent was evaporated under N<sub>2</sub> gas. Dryness was dissolved with 100  $\mu$ L mobile phase, then 20  $\mu$ L was subjected to HPLC analysis. For oral (*p.o.*) administration, the LIG/HP- $\beta$ -CD complex was redissolved in water and free LIG was suspended in 0.5 % Tween-80 with 0.5 % CMC-Na. LIG and LIG/HP- $\beta$ -CD administration were carried out *via* gastric gavage at a dose of 400 and 100 mg kg<sup>-1</sup>, respectively. Blood samples were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 h post-dosing. The procedure of blood sample treatment was the same as that described above. LIG pharmacokinetic parameters such as the peak LIG plasma concentration ( $c_{\max}$ ), the time of maximum concentration ( $t_{\max}$ ), the elimination rate constant ( $K_e$ ), the area under the curve (AUC), the half-time ( $t_{1/2}$ ) and the mean residence time (MRT) after free LIG and LIG/HP- $\beta$ -CD dosing were determined from rat plasma profiles by the noncompartmental approach using Kinetica 4.4 software.

Absolute bioavailability ( $F_{\text{abs}}$ , %) was calculated by the following formula:

$$F_{\text{abs}} (\%) = \frac{AUC_{\text{p.o.}} \text{Dose}_{\text{i.v.}}}{AUC_{\text{i.v.}} \text{Dose}_{\text{p.o.}}} \times 100 \quad (1)$$

where  $AUC_{\text{p.o.}}$  and  $AUC_{\text{i.v.}}$  are the area under the concentration-time curve after *p.o.* and *i.v.* administration, respectively.  $\text{Dose}_{\text{p.o.}}$  and  $\text{Dose}_{\text{i.v.}}$  mean the dose of LIG or LIG/HP- $\beta$ -CD following *p.o.* and *i.v.* administration, respectively.

Relative bioavailability measures the bioavailability (estimated as AUC) of LIG compared to LIG/HP- $\beta$ -CD following *p.o.* Relative bioavailability ( $F_{\text{rel}}$ ) was calculated by the following formula:

$$F_{\text{rel}} (\%) = \frac{AUC_{\text{A}} \text{Dose}_{\text{B}}}{AUC_{\text{B}} \text{Dose}_{\text{A}}} \times 100 \quad (2)$$

$AUC_{\text{A}}$  and  $AUC_{\text{B}}$  represent the area under the blood concentration-time curve of LIG/HP- $\beta$ -CD and LIG,  $\text{Dose}_{\text{A}}$  and  $\text{Dose}_{\text{B}}$  mean the dose of LIG/HP- $\beta$ -CD complex and LIG following *p.o.* administration.

## RESULTS AND DISCUSSION

### *UV-Vis absorption spectrum of the inclusion complex*

According to procedure 2.1, absorption spectra of the LIG/HP- $\beta$ -CD complex, LIG and HP- $\beta$ -CD were recorded. The absorption spectra are given in Fig. 2. The obtained results showed that HP- $\beta$ -CD had no absorption in the range 300–400 nm. A peak around

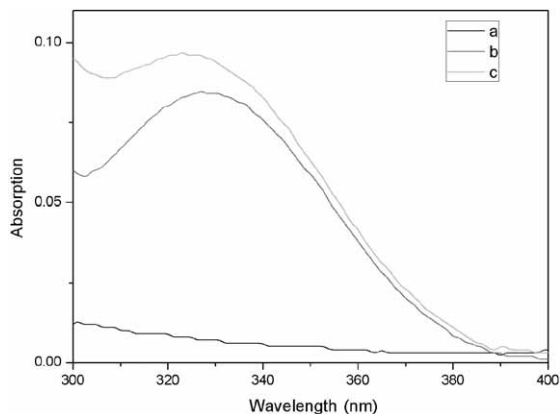


Fig. 2. UV spectra of: a) HP- $\beta$ -CD, b) LIG and c) LIG/HP- $\beta$ -CD complex.

328 nm was found on the absorption spectrum of LIG. Absorbance of the inclusion complex was higher than that of LIG alone with a simultaneous hypsochromic shift of the maximum to 324 nm. These changes were due to the interaction between LIG and HP- $\beta$ -CD, implying the inclusion complex formation (20).

#### *Stoichiometry of the inclusion complex*

According to Job's method of continuous variation, a physical parameter directly related to the concentration of the complex can be measured for a set of samples with a continuously variable molar fraction of components. Maximum concentration of the complex will be present in the sample where the molar ratio  $R$  corresponds to the complexation stoichiometry (21). The plot observed in Fig. 3 shows the maximum at a molar fraction of about 0.5, indicating that the stoichiometry of the LIG/HP- $\beta$ -CD complex was 1:1.

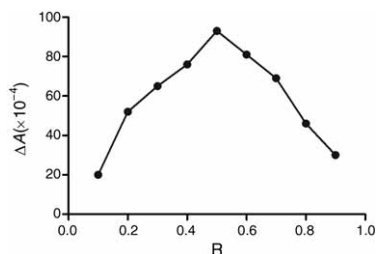


Fig. 3. Job's plot of LIG/HP- $\beta$ -CD complex.

#### *DTA*

It is known that when guest molecules are embedded in the cavity of CDs, their melting, sublimating and/or boiling points generally shift to a different temperature or disappear in the case of CD decomposition. The thermograms of LIG, HP- $\beta$ -CD, physi-

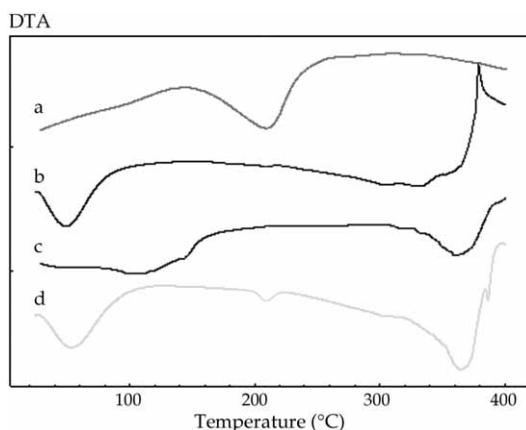


Fig. 4. DTA thermograms of: a) LIG, b) HP- $\beta$ -CD, c) LIG/HP- $\beta$ -CD complex and d) physical mixture.

cal mixture and inclusion complex are shown in Fig. 4. The DTA results demonstrate an endothermic peak for LIG (Fig. 4a) at 209.1 °C, which might be due to the decomposition of LIG. The DTA curve of HP- $\beta$ -CD alone (Fig. 4b) showed a very broad endothermic effect, which attained a maximum at around 49.2 °C corresponding to the dehydration process. Besides, the base shift around 331.5 °C may result from a degradation process of HP- $\beta$ -CD. The DTA curve of the physical mixture of LIG with HP- $\beta$ -CD (Fig. 4d) shows the persistence of the endothermic phenomenon due to loss of water, characteristic of HP- $\beta$ -CD, and the endothermic peak for LIG, indicative of the drug alone. However, the endothermic peak of LIG disappeared completely in the DTA thermogram of the inclusion complex, as can be seen from Fig. 4c. This explains the amorphous solid dispersion and the molecular encapsulation of LIG into the HP- $\beta$ -CD nanocavity (22).

### FTIR spectroscopy

Variations of the shape, shift and intensity of the FTIR absorption peaks of the guest or host molecule can provide enough information for the occurrence of inclusion. FTIR spectra of the inclusion complex and those for pure compounds as well as the physical mixture are shown in Fig. 5. The FT-IR spectrum of LIG is characterized by principal absorption peaks at 1766  $\text{cm}^{-1}$  (C=O stretching vibration); 1437, 1270 and 1051  $\text{cm}^{-1}$  (C-O stretching vibrations); 960  $\text{cm}^{-1}$  (C=C-H bending vibration of the unsaturated six-membered ring) and 704  $\text{cm}^{-1}$  (C=C-H bending vibration of the *cis*-alkene). The IR spectrum of HP- $\beta$ -CD shows prominent peaks at 3422  $\text{cm}^{-1}$  (O-H stretching vibrations); 2931  $\text{cm}^{-1}$  (C-H stretching vibrations); 1642  $\text{cm}^{-1}$  (H-O-H bending vibrations); 1038 and 1084  $\text{cm}^{-1}$  (C-H, C-O stretching vibrations). It can be seen that the spectrum of the physical mixture is essentially a combination of LIG and HP- $\beta$ -CD, indicating that a physical mixture does not lead to inclusion. However, there were obvious changes in the FTIR spectra after the LIG/HP- $\beta$ -CD complex was formed. The band at 1270  $\text{cm}^{-1}$  corresponding to C-O stretching vibrations of the lactone ring disappeared in the complex. The band at 1766  $\text{cm}^{-1}$

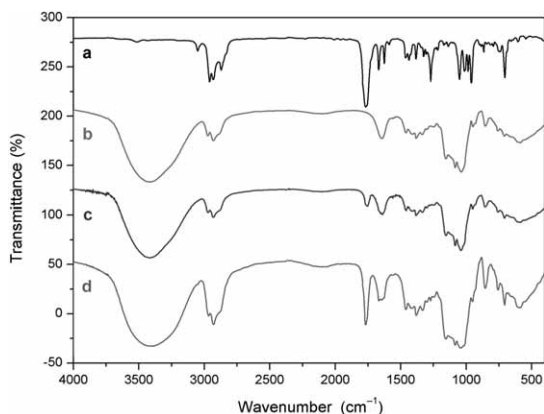


Fig. 5. FTIR spectra of: a) LIG, b) HP- $\beta$ -CD, c) LIG/HP- $\beta$ -CD complex and d) physical mixture.

corresponding to C=O stretching vibration shifted to  $1752\text{ cm}^{-1}$  and the intensity decreased. The observed changes in the FTIR spectra of LIG complexed with HP- $\beta$ -CD are due to a restriction of the vibration of the LIG molecule upon its encapsulation into the HP- $\beta$ -CD cavity (23). Therefore, the FTIR spectroscopy result indicated that the inclusion complex of LIG with HP- $\beta$ -CD was obtained.

### Stability studies

Inclusion complexation with CDs can affect the physical and chemical properties of guest molecules and usually leads to retardation of degradation processes (24). As shown in Fig. 6a, within 6 days under light, free LIG underwent severe degradation of 77.9 %, whereas the LIG remaining in the inclusion complex was 77.8 %. Although the effect of light on the inclusion complex was still significant, the stability of LIG against light was greatly enhanced when it formed the inclusion complex with HP- $\beta$ -CD.

During 6 days, degradation of free LIG occurred rapidly at 4 and 30 °C (Figs. 6b and c) and reached the degradation percentage of 31.80 and 89.09 %, respectively. However, the inclusion complex remained stable for 6 days at 4 and 30 °C. At 50 °C, LIG degraded completely within 5 days. Although LIG remaining in the inclusion complex reduced over time at 50 °C, the reduction speed was much lower than that of pure LIG. These results strongly suggest that inclusion complexation afforded an efficient protection for LIG. Thus, the stability of LIG against elevated temperature was effectively increased through its complexation with HP- $\beta$ -CD.

### Pharmacokinetics in rat plasma

The HPLC method for the determination of LIG in rat plasma has been developed and validated over a concentration range of  $20\text{--}1000\text{ ng mL}^{-1}$ . The calibration curve for LIG was prepared as  $y = 0.0012x - 0.0266$  ( $R = 0.9968$ ). The RSD of LIG for inter-day and intra-day precision and accuracy at low, medium and high concentration (20, 200 and



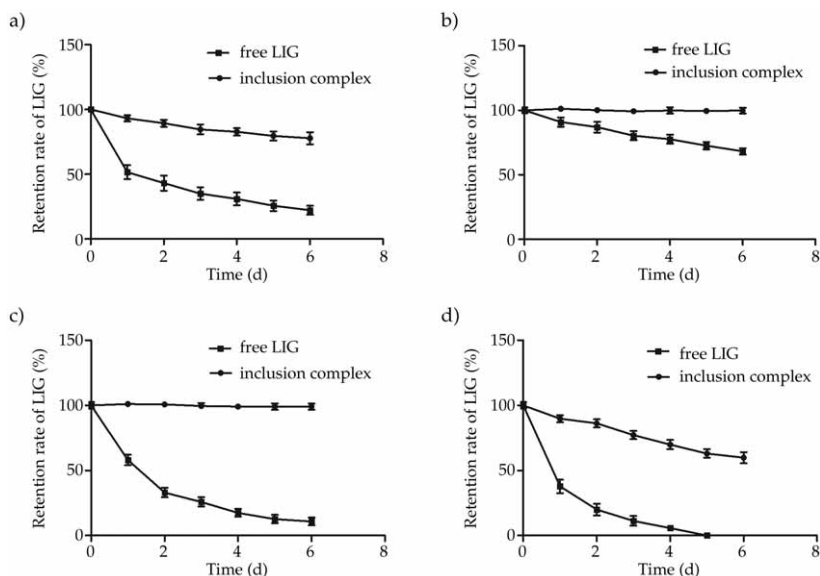


Fig. 6. Stability of LIG and inclusion complex under: a) light and at temperature of b) 4, c) 30 and d) 50 °C.

4000 ng mL<sup>-1</sup>) was less than 11.2 %. LIG recoveries at the above mentioned concentrations were 84.1, 86.2 and 93.5 %, respectively. The *LOQ* and *LOD* for LIG were 20 and 10 ng mL<sup>-1</sup>, respectively.

Table I. Pharmacokinetic parameters of LIG administered at different doses and different preparations in rats

Pharmacokinetic parameters	Lig(20 mg kg <sup>-1</sup> <i>i.v.</i> )	Lig(400 mg kg <sup>-1</sup> <i>p.o.</i> )	Lig/HP- $\beta$ -CD (100 mg kg <sup>-1</sup> <i>p.o.</i> )
<i>A</i> (ng mL <sup>-1</sup> )	2458.55 ± 782.42	2764.92 ± 1357.72	1886.86 ± 265.65
<i>K<sub>e</sub></i> (h <sup>-1</sup> )	1.44 ± 0.35	1.17 ± 0.33	0.70 ± 0.25
<i>K<sub>a</sub></i> (h <sup>-1</sup> )	150.47 ± 14.52	2.65 ± 0.73	4.36 ± 0.97
<i>t</i> <sub>1/2</sub> (h)	0.51 ± 0.15	0.62 ± 0.14	1.07 ± 0.31
<i>MRT</i> (h)	1.12 ± 0.16	2.11 ± 0.23	2.75 ± 0.20
<i>c</i> <sub>max</sub> (ng mL <sup>-1</sup> )	3244.26 ± 484.14	2640.55 ± 483.91	1979.57 ± 666.83
<i>T</i> <sub>max</sub> (h)	0.08 ± 0.00	0.50 ± 0.00	0.50 ± 0.00
<i>Vc/F</i> (mg ng <sup>-1</sup> mL <sup>-1</sup> )	0.01 ± 0.00	0.17 ± 0.06	0.05 ± 0.01
<i>AUC<sub>t</sub></i> (h ng mL <sup>-1</sup> )	1912.35 ± 141.19	2857.98 ± 522.94	3428.95 ± 569.28
<i>AUC<sub>i</sub></i> (h ng mL <sup>-1</sup> )	1990.68 ± 110.61	2877.11 ± 518.85	3465.72 ± 573.56
<i>AIC</i>	40.37 ± 3.76	70.39 ± 2.77	65.01 ± 5.17
<i>F</i> <sub>abs</sub> (%)	100	7.47	35.86

Each value represents the mean ± SD, *n* = 4.

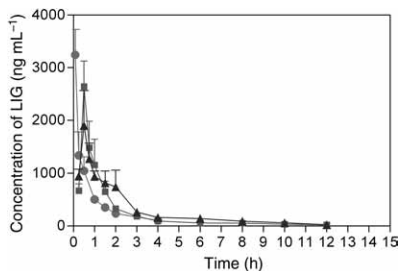


Fig. 7. Mean ( $\pm$  SD) plasma concentration-time profiles of LIG after intravenous administration of free LIG at a dose of  $20 \text{ mg kg}^{-1}$  (●), oral administration of free LIG at a dose of  $400 \text{ mg kg}^{-1}$  (■) and LIG/HP- $\beta$ -CD complex at a dose of  $100 \text{ mg kg}^{-1}$  (▲),  $n = 4$ .

The mean plasma concentration *vs.* time profiles of LIG administered at different doses and different preparations in rats are shown in Fig. 7. The plasma drug concentration in rats after receiving an *i.v.* dose of  $20 \text{ mg kg}^{-1}$  LIG increased rapidly and with an average maximal concentration ( $c_{\text{max}}$ ) of  $3244.26 \pm 484.14 \text{ ng mL}^{-1}$  and an average time to  $c_{\text{max}}$  ( $t_{\text{max}}$ ) of  $0.08 \pm 0.00 \text{ h}$ . After oral administration of  $400 \text{ mg kg}^{-1}$  LIG and  $100 \text{ mg kg}^{-1}$  LIG/HP- $\beta$ -CD complex, the plasma drug concentration in rats also increased rapidly and reached a  $c_{\text{max}}$  of  $2640.55 \pm 483.91 \text{ ng mL}^{-1}$  and  $1979.57 \pm 666.83 \text{ ng mL}^{-1}$  at the same time ( $0.50 \pm 0.00 \text{ h}$ ).

LIG/HP- $\beta$ -CD showed high plasma concentrations in rats compared to free LIG. The absolute bioavailability ( $F_{\text{abs}}\%$ ) values of LIG in the rats treated with LIG/HP- $\beta$ -CD were significantly higher (35.9 %) than those of free LIG (7.5 %). Relative bioavailability ( $F_{\text{rel}}\%$ ) of LIG/HP- $\beta$ -CD was about 530 % and there was an about 4.8-fold increase in apparent bioavailability. It was reported that the oral bioavailability of LIG was only 2.6 % in rats (25). The results obtained from the pharmacokinetic study of LIG after oral administration clearly indicate that inclusion of LIG into HP- $\beta$ -CD could increase both the extent and the rate of its oral absorption. Since LIG is a promising neuroprotective agent for preventing and treating ischemic disorders and AD, the current study reveals that HP- $\beta$ -CD may be effectively utilized for the inclusion of LIG in designing appropriate formulations to meet clinical needs.

## CONCLUSIONS

In conclusion, this study showed that LIG was capable of forming an inclusion complex with HP- $\beta$ -CD in the stoichiometric ratio of 1:1. The structure of the inclusion complex was characterized by UV-Vis spectroscopy, DTA and FTIR spectroscopy. Stability studies indicated that HP- $\beta$ -CD can greatly improve the stability of LIG against temperature and light in the form of an inclusion complex. Further, the oral bioavailability of LIG in rats was greatly enhanced upon its complexation with HP- $\beta$ -CD. These results strongly suggest that LIG/HP- $\beta$ -CD complexation can be of great use in increasing the stability and biological efficacy of LIG.

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## REFERENCES

1. H. Y. Peng, J. R. Du, G. Y. Zhang, X. Kuang, Y. X. Liu, Z. M. Qian and C. Y. Wang, Neuroprotective effect of Z-ligustilide against permanent focal ischemic damage in rats, *Biol. Pharm. Bull.* 30 (2007) 309–312; DOI: JST.JSTAGE/bpb/30.309 [pii].
2. J. Sun, B. K. Tan, S. H. Huang, M. Whiteman and Y. Z. Zhu, Effects of natural products on ischemic heart diseases and cardiovascular system, *Acta Pharmacol. Sin.* 23(2002) 1142–1151.
3. J. Y. Zhan, K. Y. Zheng, K. Y. Zhu, C. W. Bi, W. L. Zhang, C. Y. Du, Q. Fu, T. T. Dong, R. C. Choi, K. W. Tsim and D. T. Lau, Chemical and biological assessment of *Angelicae Sinensis Radix* after processing with wine: an orthogonal array design to reveal the optimized conditions, *J. Agric. Food Chem.* 59 (2011) 6091–6098; DOI: 10.1021/jf200728e.
4. K. Y. Zheng, R. C. Choi, A. W. Cheung, A. J. Guo, C. W. Bi, K. Y. Zhu, Q. Fu, Y. Du, W. L. Zhang, J. Y. Zhan, R. Duan, D. T. Lau, T. T. Dong and K. W. Tsim, Flavonoids from *Radix Astragali* induce the expression of erythropoietin in cultured cells: a signaling mediated via the accumulation of hypoxia-inducible factor-1 $\alpha$ , *J. Agric. Food Chem.* 59 (2011) 1697–1704; DOI: 10.1021/jf104018u.
5. X. M. Wu, Z. M. Qian, L. Zhu, F. Du, W. H. Yung, Q. Gong and Y. Ke, Neuroprotective effect of ligustilide against ischemia-reperfusion injury via up-regulation of erythropoietin and down-regulation of RTP801, *Br. J. Pharmacol.* 164 (2011) 332–343; DOI: 10.1111/j.1476-5381.2011.01337.x.
6. X. Kuang, J. R. Du, Y. X. Liu, G. Y. Zhang and H. Y. Peng, Postischemic administration of Z-ligustilide ameliorates cognitive dysfunction and brain damage induced by permanent fore-brain ischemia in rats, *Pharmacol. Biochem. Behav.* 88 (2008) 213–221; DOI: 10.1016/j.pbb.2007.08.006.
7. X. Kuang, J. R. Du, Y. S. Chen, J. Wang and Y. N. Wang, Protective effect of Z-ligustilide against amyloid beta-induced neurotoxicity is associated with decreased pro-inflammatory markers in rat brains, *Pharmacol. Biochem. Behav.* 92 (2009) 635–641; DOI: 10.1016/j.pbb.2009.03.007.
8. F. Cui, L. Feng and J. Hu, Factors affecting stability of z-ligustilide in the volatile oil of *radix angelicae sinensis* and *ligusticum chuanxiong* and its stability prediction, *Drug Dev. Ind. Pharm.* 32 (2006) 747–755; DOI: 10.1080/03639040500529101.
9. S. Pinnamaneni, N. G. Das and S. K. Das, Formulation approaches for orally administered poorly soluble drugs, *Pharmazie* 57 (2002) 291–300.
10. J. Szejtli, Introduction and general overview of cyclodextrin chemistry, *Chem. Rev.* 98 (1998) 1743–1754; DOI: cr970022c [pii].
11. L. Szente and J. Szejtli, Highly soluble cyclodextrin derivatives: chemistry, properties, and trends in development, *Adv. Drug Deliv. Rev.* 36 (1999) 17–28; DOI: S0169-409X(98)00092-1 [pii].
12. S. Gould and R. C. Scott, 2-Hydroxypropyl-beta-cyclodextrin (HP-beta-CD): a toxicology review, *Food Chem. Toxicol.* 43 (2005) 1451–1459; DOI: 10.1016/j.fct.2005.03.007.

13. G. E. Granero, M. M. Maitre, C. Garnero and M. R. Longhi, Synthesis, characterization and in vitro release studies of a new acetazolamide-HP-beta-CD-TEA inclusion complex, *Eur. J. Med. Chem.* **43** (2008) 464–470; DOI: 10.1016/j.ejmech.2007.03.037.
14. B. Yang, J. Lin, Y. Chen and Y. Liu, Artemether/hydroxypropyl-beta-cyclodextrin host-guest system: characterization, phase-solubility and inclusion mode, *Bioorg. Med. Chem.* **17** (2009) 6311–6317; DOI: 10.1016/j.bmc.2009.07.060.
15. C. Yuan, Z. Y. Jin, X. M. Xu, H. N. Zhuang and W. Y. Shen, Preparation and stability of the inclusion complex of astaxanthin with hydroxypropyl-beta-cyclodextrin, *Food Chem.* **109** (2008) 264–268; DOI: 10.1016/j.foodchem.2007.07.051.
16. Y. Yu, J. R. Du, C. Y. Wang and Z. M. Qian, Protection against hydrogen peroxide-induced injury by Z-ligustilide in PC12 cells, *Exp. Brain Res.* **184** (2008) 307–312; DOI: 10.1007/s00221-007-1100-3.
17. Q. F. Zhang, Z. T. Jiang, Y. X. Guo and R. Li, Complexation study of brilliant cresyl blue with beta-cyclodextrin and its derivatives by UV-vis and fluorospectrometry, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **69** (2008) 65–70; DOI: 10.1016/j.saa.2007.03.009.
18. L. M. Pinto, L. F. Fraceto, M. H. Santana, T. A. Pertinhez, S. O. Junior and de E. Paula, Physico-chemical characterization of benzocaine-beta-cyclodextrin inclusion complexes, *J. Pharm. Biomed. Anal.* **39** (2005) 956–963; DOI: 10.1016/j.jpba.2005.06.010.
19. X. Ge, Z. Huang, S. L. Tian, Y. L. Huang and C. Z. Zeng, Complexation of carbendazim with hydroxypropyl-beta-cyclodextrin to improve solubility and fungicidal activity, *Carbohydr. Polym.* **89** (2012) 208–212; DOI: 10.1016/j.carbpol.2012.02.072.
20. P. Gornas, G. Neunert, K. Baczynski and K. Polewski, Beta-cyclodextrin complexes with chlorogenic and caffeic acids from coffee brew: Spectroscopic, thermodynamic and molecular modelling study, *Food Chem.* **114** (2009) 190–196; DOI: 10.1016/j.foodchem.2008.09.048.
21. D. R. de Araujo, S. S. Tsuneda, C. M. S. Cereda, F. Del G. F. Carvalho, P. S. C. Preté, S. A. Fernandes, F. Yokaichiya, M. K. K. D. Franco, I. Mazzaro, L. F. Fraceto, A. de F. A. Braga and E. de Paula, Development and pharmacological evaluation of ropivacaine-2-hydroxypropyl-beta-cyclodextrin inclusion complex, *Eur. J. Pharm. Sci.* **33** (2008) 60–71; DOI: 10.1016/j.ejps.2007.09.010.
22. P. Mura, E. Adragna, A. M. Rabasco, J. R. Moyano, J. I. Perez-Martinez, A. J. Arias and J. M. Gines, Effects of the host cavity size and the preparation method on the physicochemical properties of ibuprofen-cyclodextrin systems, *Drug Dev. Ind. Pharm.* **25** (1999) 279–287; DOI: 10.1081/DDC-100102172.
23. E. E. M. Eid, A. B. Abdul, F. E. O. Suliman, M. A. Sukari, A. Rasedee and S. S. Fatah, Characterization of the inclusion complex of zerumbone with hydroxypropyl-beta-cyclodextrin, *Carbohydr. Polym.* **83** (2011) 1707–1714; DOI: 10.1016/j.carbpol.2010.10.033.
24. Y. Y. Hwang, D. C. Shin, Y. S. Nam and B. K. Cho, Characterization, stability, and pharmacokinetics of sibutramine/beta-cyclodextrin inclusion complex, *J. Ind. Eng. Chem.* **18** (2012) 1412–1417; DOI: 10.1016/j.jiec.2012.01.046.
25. R. Yan, N. L. Ko, S. L. Li, Y. K. Tam and G. Lin, Pharmacokinetics and metabolism of ligustilide, a major bioactive component in Rhizoma Chuanxiong, in the rat, *Drug Metab. Dispos.* **36** (2008) 400–408; DOI: 10.1124/dmd.107.017707.