School of Ocean[1], Shandong University, Weihai; School of Pharmaceutical Sciences[2], Shandong University, Jinan, China

Enhanced bioavailability of raloxifene hydrochloride via dry suspensions prepared from drug/HP-β-cyclodextrin inclusion complexes

Rong Lu[1], Shan Liu[1], Qilin Wang[1], Xia Li[1,2]

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Xia Li, School of Ocean, Shandong University, 180 West Wenhua Rd., Weihai 264209, China. xiali@sdu.edu.cn

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This study aimed to develop a dry suspension formulation of raloxifene (RLX) using its HP-β-cyclodextrin inclusion complexes to enhance the oral bioavailability. Dry suspensions loading RLX/HP-β-cyclodextrin inclusion complexes (RLX-HICs) were prepared by solvent evaporation followed by a standard wet granulation process. The inclusion complexes were characterized by scanning electron microscopy, differential scanning calorimetry, and Fourier transform infrared spectroscopy. The features of dry suspensions such as dispersibility, flowability and dissolution were compared with conventional suspensions. Dry suspensions containing RLX-HICs dramatically increased the dissolution of RLX. Pharmacokinetic studies in rats showed that dry suspensions with RLX-HICs significantly enhanced the oral bioavailabilities of RLX. The absolute and relative bioavailabilities were up to 13.04% and 413.97% compared with the solution formulation (*i.v.*) and conventional suspensions (*i.g.*), respectively. The bioavailability improvement for dry suspensions with RLX-HICs can be attributed to improved dissolution and physiochemical properties of RLX, by which the overall absorption was enhanced. Dry suspensions prepared from RLX-HICs may be an attractive formulation for the oral delivery of RLX.

1. Introduction

Oral drug delivery remains to be the preferred route of administration due to convenience and compliance. Nevertheless, oral drug delivery is often fraught with difficulties associated with multiple factors, such as aqueous solubility, membrane permeability, gastrointestinal (GI) residence time, and presystemic metabolism of drug (Huang et al. 2009). For most of insoluble drugs, the rate and extent of oral absorption (bioavailability) is generally governed by the dissolution rate of drug in the gastrointestinal (GI) tract (Lobenberg and Amidon 2000). In addition to dissolution, the biochemical characteristics of drug (e.g., GI instability and susceptibility to efflux pump) also play negative roles in bioavailability, especially for BCS IV drugs. An approach that can simultaneously improve the dissolution and insoluble nature of drugs is required to overcome the absorptive barriers.

Raloxifene hydrochloride (RLX, Fig. 1) is a selective estrogen receptor regulator used to prevent osteoporosis in postmenopausal women, and also to remedy the estrogen-dependent breast tumors (Fujiwara et al. 2014; Gizzo et al. 2013). Oral therapy with RLX is less effective attributable to poor bioavailability that results in an inadequate therapeutic blood level. The insoluble nature and significant first-pass effect are considered as the main causes of low systemic RLX concentration via the oral route (Aditya et al. 2014). The absolute bioavailability was reported to be approximately 2%after oral administration (Heringa, 2003; Hochner-Celnikier, 1999). To improve the dissolution and bioavailability of RLX, several strategies have been attempted, including solid dispersions (Tran et al. 2013b), self-emulsifying delivery systems (Thakkar et al. 2011), solid lipid nanoparticles (Ravi et al. 2014), and inclusion complexes (Wempe et al. 2008). However, it is noted that RLX is not only poorly soluble in water, but also in oils and lipids. RLX possesses a water solubility of ∼300 μg/mL (hydrochloride form), and is highly incompatible with frequently used lipid excipients. The insoluble nature of RLX always leads to failure of formulations development, such as liposomes, lipid nanoparticles, and micro/nanoemulsions. Apparentdrug precipitation from nanocarriers upon storage builds the primary obstacle to engineering nanoparticle-based delivery systems of RLX. In

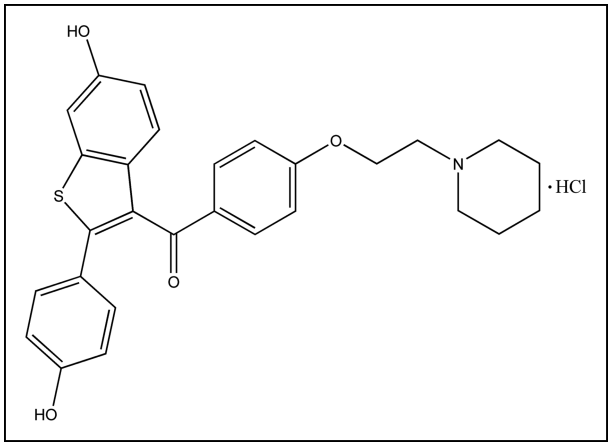

Fig. 1: Chemical structure of raloxifene hydrochloride.

this respect, some innovative solid formulations such as those containing solid dispersions (Cho et al. 2014) or cyclodextr in inclusion complexes (Zhang et al. 2009) can make up for the shortcomings of drug precipitation. Although solid dispersions have the ability to significantly enhance the dissolution of poorly water-soluble drugs (Zhang et al. 2008), they are generally feeble in amelioration of unfavorable properties of them. In contrast, inclusion complexes possess an additional merit in improving drug properties.

Drug/cyclodextrin conjugates are pharmaceutically defined as inclusion complexes where the drug (guest molecule) is encapsulated into the cavity of cyclodextrin (host molecule). Besides, to improve dissolution, inclusion complexes are able to enhance the stability of drug, mask the unpleasant taste, reduce the GI degradation, and inhibit drug efflux (Miller et al. 2007; Rong et al. 2014). It was shown that intestinal glucuronidation had a more significant impact on the oral bioavailability of RLX than hepatic glucuronidation in humans (Mizuma 2009). Lee's group demonstrated that pharmaceutical excipients such as Tween 80and PVP k30 could reduce the *in vitro* metabolism of RLX (Kim et al. 2009). Cyclodextrins like HP-β-cyclodextrin(HP-β-CD) may be helpful to reduce presystemic metabolism and enhance the bioavailability of RLX. However, solid formulations containing RLX/HP-β-CD inclusion complexes (RLX-HICs) as well as the effect of complexation on oral absorption of RLX have not been investigated.

In this study, we developed a dry suspension formulations containing RLX-HICs with the aim of convenient administration and bioavailability enhancement. Dry suspensions with RLX-HICs were prepared by prefabrication of RLX-HICs followed by granulation with selective excipients. RLX-HICs were characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and Fourier transform infrared spectroscopy (FTIR). The suitability of a formulation with RLX-HICs was evaluated by dissolution and bioavailability studies.

2. Investigations, results and discussion

2.1. Solubility diagram of RLX/HP-β-CD

Figure 2 shows the phase solubility diagram of RLX *versus* HP-β-CD with increasing concentration in water. The complexation between RLX and HP-β-CD was extrapolated to be an A_L-type by linear fitting with a correlation coefficient of 0.9825. This type of plot indicated a stoichiometric rate of 1:1 molecular complexation between RLX and HP-β-CD.The aqueous solubility of RLX determined in the absence of HP-β-CD was 322.7 μg/mL (6.32×10^{-4} mol/L). Thus, the calculated complex formation constant (K_f) was 920.9 L/M, demonstrating a stable complexation. The phase solubility study suggested that it was feasible to utilize HP-β-CD to encapsulate and solubilize RLX.

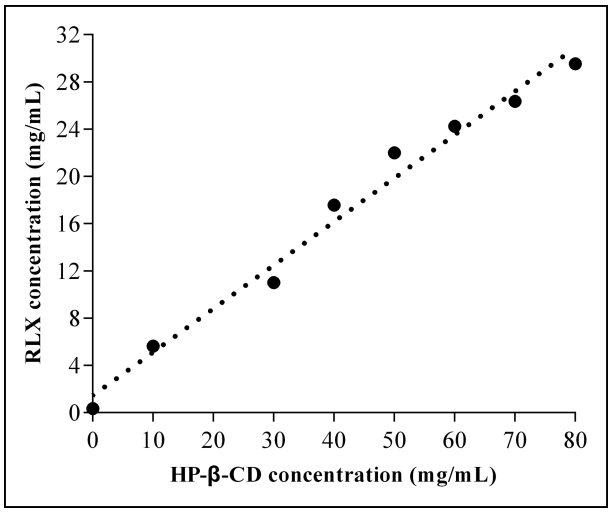

Fig. 2: Phase solubility diagram plotted with concentration of raloxifene against increasing concentrations of HP-β-cyclodextrin in water (pH 6.8).

2.2. Preparation and characterization of RLX-HICs

Various techniques can be used to prepare inclusion complexes, such as saturated aqueous solution, kneading, freeze/spray-drying, co-precipitation,and supercritical fluid processes (Hassan et al. 2007; Miller et al. 2007). In the present study, co-precipitation from drug/cyclodextr in solution was selected to prepare RLX-HICs. This technique is facile and of high performance in manufacturing. The resultant RLX-HICs displayed a yellow transparent appearance that was significantly different from the raw material of RLX, suggesting a molecular entrapment of RLX intothe HP-β-CD matrix. Also, SEM micrographs provided some information on the morphology changes of substance phase (Fig. 3). Obvious crystalline structure was provided with pure RLX (Fig. 3A), while HP-β-CD exhibited anamorphous state (Fig. 3B).Of note, there were no crystallites associated with RLX or heterogeneous phases observable in RLX-HICs (Fig. 3C), demonstrating the complete entrapment of RLX into the cavities of HP-β-CD.

Figure 4 shows the thermogram changes of RLX before and after complexation. The endothermic peak at 245 °C denoted the melting point of RLX (Fig. 4A). HP-β-CDshowed no endothermic events as subjected to DSC scanning due to its amorphous nature (Fig. 4B). The the rmogram of physical mixture was more like an overlap of endothermic peaks from RLX and HP-β-CD (Fig. 4C). However, the melting point peak of RLX disappeared in the DSC curve of RLX-HICs (Fig. 4D), indicating the absence of RLX crystallinity. It lent a strong support to the notion that RLX has been incorporated into the cavity of HP-β-CD.

FTIR spectra of RLX, HP-β-CD, physical mixture, and RLX-HICs are shown in Fig. 5. There were significant changes in the

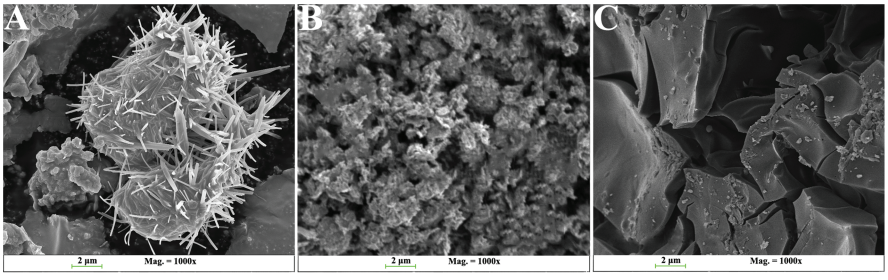

Fig. 3: SEM photographs of raloxifene (A), HP-β-cyclodextrin (B), and raloxifene/HP-β-cyclodextrininclusion complexes (C).

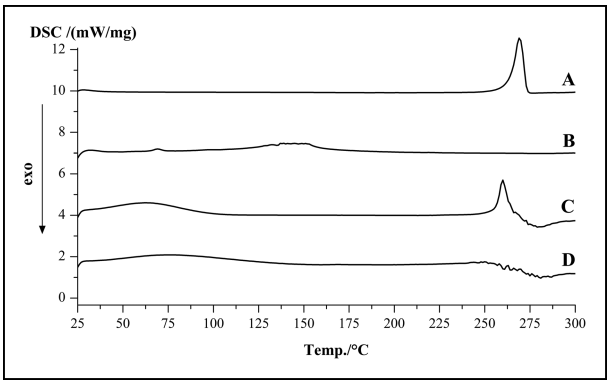

Fig. 4: DSC thermograms of raloxifene (A), HP-β-cyclodextrin (B), physical mixture (C), and raloxifene/HP-β-cyclodextrin inclusion complexes (D).

characteristic bands of the RLX spectrum, revealing an alteration of molecule micromilieu. The diagnostic peaks were located on2752.12, 2694.23,and 1642.14 cm^{-1}, corresponding to the stretching vibrations of-CH_2- (ν_s)and –C_3N (ν_s), respectively. RLX-associated peaks for RLX and physical mixture were nearly identical. However, those diagnostic peaks, as marked by gray lines, vanished or shifted in position in the FTIR spectrum of RLX-HICs. Changes in characteristic peaks indicated that there were molecular interactions between RLX and HP-β-CD. This might be ascribable to the effect of inclusion or association.

FTIR is the frequently used tool to investigate the complexation process of drug and cyclodextrin (Aloisio et al. 2014; Lu et al. 2012). From the FTIR spectra, it could be hypothesized that the piperidine ring and adjacent ethoxy moiety of RLX were included into the cavity of HP-β-CD. In the FTIR absorption of methylene and carbon-nitrogen bond, pertaining to the ethoxy and piperidine, notable changes occurred. The former disappeared and the latter shifted up in the case of RLX-HICs. The proposed inclusion process of RLX with HP-β-CDwas schematically illustrated in Fig. 6.

2.3. Preparation and characterization of dry suspensions

Oral dry suspensions refer to a ready-to-use solid dosage form that can be immediately suspended in water before administration. Dry suspensions with RLX-HICs were composed of

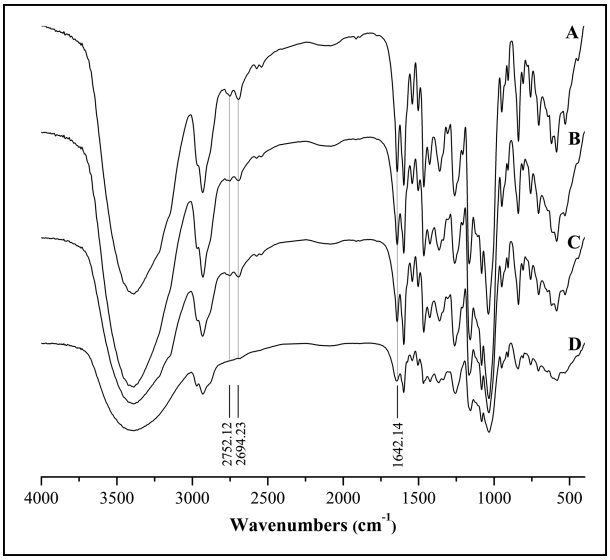

Fig. 5: FTIR spectra of raloxifene (A), HP-β-cyclodextrin (B), physical mixture (C), and raloxifene/HP-β-cyclodextrin inclusion complexes (D).

essential excipients, including carboxymethyl starch sodium (as a suspending agent), hydroxypropyl cellulose (as a dispersing agent), and talc (as a lubricant). As a parallel formulation, dry suspensions with RLX contained the same excipients except for sucrose that was used to replace HP-β-CD. The common wet granulation with ethanol as a binder was adopted to prepare the dry suspensions. In contrast to the technique of simple mixing, the powders produced by wet granulation are superior in flow ability and dispersibility. Due to the solubility for RLX, RLX-HICs and hydroxypropyl cellulose in ethanol, it was required by the technique to adsorb ethanol with carboxymethyl starch sodium before hand to prevent the agglutination of materials upon granulation. The technique that utilize ethanol-imbibed carboxymethyl starch sodium to fabricate granules with the remaining compositions allowed the granulation to be processed smoothly.

Characteristic parameters of two dry suspensions are listed in Table 1. The resultant dry suspensions with RLX-HICs exhibited an excellent dispersibility compared to dry suspensions with RLX. It was assumed that increased aqueous solubility and wettability were the underlying causes, as reflected by the case that the increase of wettability could result in enhanced dissolution of solid dispersions (Lu et al. 2014). Similarly, the redispersibility upon sedimentation correlated well with the hydrophilic nature of the material. Pure RLX possesses a poor water-solubility and wettability. The unpleasant properties of RLX easily lead to its separation from the dispersants upon dispersion with water because of differential solubility. This was the reason for weak redispersibility provided by dry suspensions with RLX. Reversely, the flowability of the formulation containing RLX-HICs decreased somewhat in comparison with the formulation containing RLX. The former had a repose angle of 35.8°, while it was 27.4 for the latter. Generally, hydrophobic substances such as talc and magnesium stearate have a good fluidity, since they are lowlyglutinous andhygroscopic. The alteration of powder flowability could be attributed to the physiochemical modification of RLX (e.g., hydroscopicity and contact angle), as a consequence of complexation. It was also a sign that the product from the co-precipitation of RLX and HP-β-CD was significantly different from the raw material of RLX.

The drug contents of dry suspensions with RLX-HICs and RLX were 5.37% and 5.62%, respectively. It was uniform in drug content among batches (RSD less than 2%), demonstrating an acceptable process reproducibility. The general related substances were less than 0.2% in cases of two dry suspensions. As compared with the raw material, there were no significant differences. The negligible change in related substances revealed that the preparative processes (e.g., evaporation, shearing, and drying)would not produce new impurities.

2.4. In vitro dissolution

The dissolution profiles of RLX, conventional suspensions, and RLX-HICs-contained suspensions in waterare shown in Fig. 7. The raw material of RLX yielded a terribly slow dissolution, and the accumulative dissolution was just 0.95% within 60 min. The dissolution rate of RLX was largely limited by its aqueous solubility and wettability. Likewise, conventional dry suspensions exhibited a slow dissolution with a dissolution percentage of 7.04% at 60 min. Although the dissolution rate was higher than that of pure RLX owing to the increase of dispersibility, the dissolution was fairly inactive. The phenomenon suggested that RLX dissolution was primarily dominated by the intrinsic solubility, and followed by the dispersity. Compared to conventional suspensions, the dissolution of RLX in the case of dry

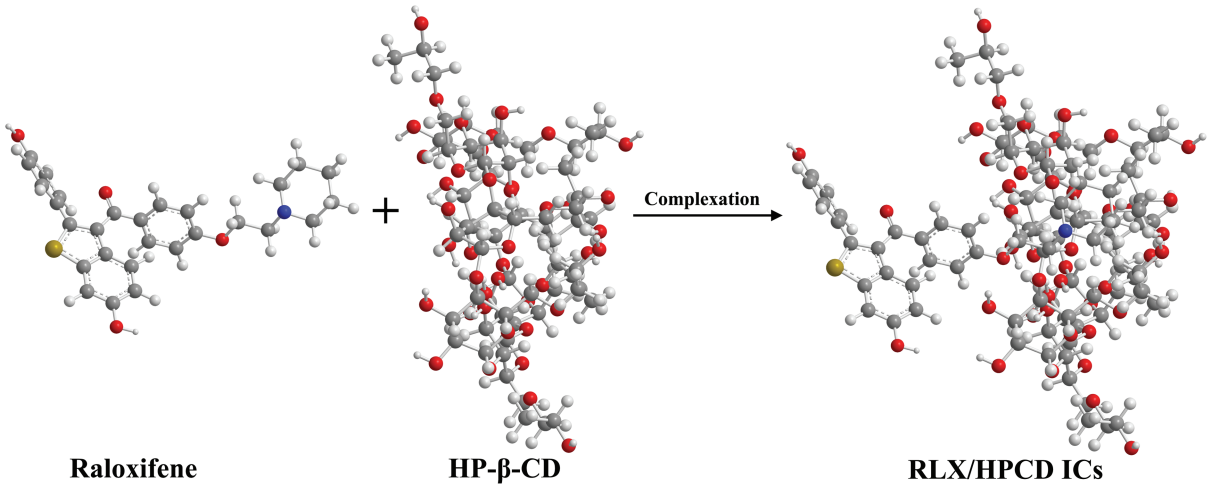

Fig. 6: Molecular modeling of inclusion process of raloxifene with HP-β-cyclodextrin.

Table 1: Characterization of dry suspensions loading RLX-HICs and conventional dry suspensions loading RLX

Formulation	Dispersibility	Redispersibility upon sedimentation	Repose angle (°)	Drug loading (%)	Related substances (%)
DS-RLX-HICs	Good	Good	35.8 ± 2.4	5.37 ± 0.47	0.09 ± 0.07
DS-RLX	Medium	Weak	27.4 ± 1.3	5.62 ± 0.36	0.11 ± 0.05

DS-RLX-HICs: dry suspensions loading RLX-HICs; DS-RLX: dry suspensions loading RLX;

suspensions with RLX-HICs was strikingly enhanced. The dissolution rate was up to 72.12% at 5 min, and almost completed at 15 min.

Coarsely dispersed drugs are unable to adequately approach the mucosae and readily removed from the GI tract, resulting in reduced oral bioavailability. Fine dispersion systemshave proved theirpotentialin enhancing the dissolution and bioavailability of poorly water-soluble drugs, such as solid dispersions (Ramadhani et al. 2014) and nanocrystals (Fu et al. 2013). Cyclodextrin inclusion complexes improved the aqueous solubility of RLX, and it further enhanced the dissolution by formulating them into highly dispersed suspensions. Besides dissolution enhancement, dry suspensions containing RLX-HIC spossess variousfeatures of cyclodextrin inclusion complexes, e.g., metabolism inhibition (Hirayama et al. 1995), taste masking (Arima et al. 2012), and absorption promotion (Loftsson et al. 2007).

2.5. Enhanced bioavailability of RLX

Plasma RLX concentration *versus* time curves are presented in Fig. 8 after intragastric (*i.g.*) dosing of suspensions with RLX-HICs or RLX and intravenous (*i.v.*) injection of RLX solution. Main pharmacokinetic parameters derived from non-compartmental model are shown in Table 2. There were significant differences in pharmacokinetics between dry suspensions with RLX-HICs and RLX. The peak concentration (C_{max}) of RLX-HICs formulation reachedup to 0.405 μg/mL, whereas it just was 0.106 μg/mL for RLX formulation. The peak time (T_{max}) of RLX-HICs formulation appeared more promptly, indicating faster absorption of RLX-HICs than coarsely dispersed RLX. Conventional dry suspensions possessed a relatively long elimination half-life ($T_{1/2}$)due to slow absorption, compared to the suspensions with RLX-HICs. This was related to

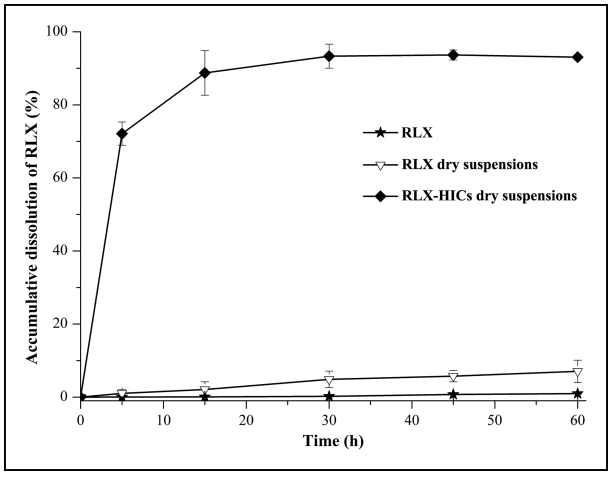

Fig. 7: Dissolution profiles of raloxifene, conventional dry suspensions loading raloxifene, and dry suspensions loading raloxifene/HP-β-cyclodextrin inclusion complexes.

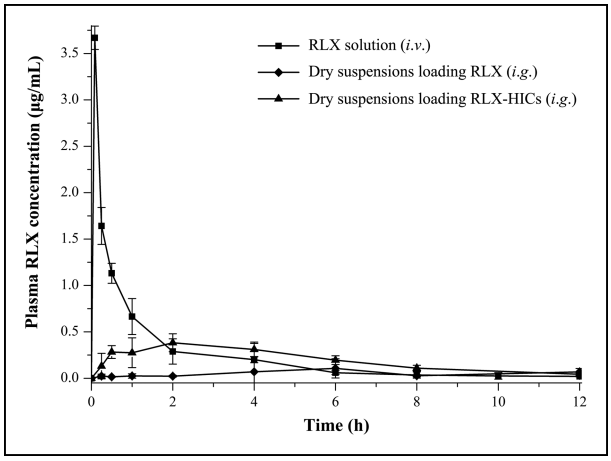

Fig. 8: Plasma raloxifene concentration-time profiles after administration of conventional dry suspensions loading raloxifene (*i.g.*), dry suspensions loading raloxifene/HP-β-cyclodextrin inclusion complexes (*i.g.*) and raloxifene solution (*i.v.*) to rats at a dose of 30 mg/kg (mean ± SD, $n = 5$).

Table 2: Pharmacokinetic parameters of dry suspensions loading RLX-HICs or RLX (*i.g.*, 30 mg/kg) and of RLX solution (*i.v.*, 5 mg/kg) in rats ($n = 5$, mean ± SD)

Parameters	DS-RLX-HICs	DS-RLX	RLX solution
C_{max} (μg/mL)	0.405 ± 0.052**	0.106 ± 0.061	3.672 ± 0.133
T_{max} (h)	2.100 ± 0.824**	6.017 ± 2.34	0.083
$T_{1/2}$ (h)	2.765 ± 0.652**	16.526 ± 3.867	3.535 ± 1.647
$AUC_{0\text{-}t}$ (μg·h/mL)	2.344 ± 0.281**	0.567 ± 0.219	2.997 ± 0.219
ABA(%)	13.04	3.15	
RBA(%)	413.97	/	

DS-RLX-HICs: dry suspensions loading RLX-HICs; DS-RLX: dry suspensions loading RLX;*AUC*: area under the plasma RLX concentration-time curve; *ABA* and *RBA*: absolute and relative bioavailability, respectively. One-way ANOVA, $^{**}p < 0.01$, compared with the reference of RLX dry suspensions.

the lower plasma RLX concentration and elongatedabsorption phase. Dry suspensions with RLX-HIC sproduced remarkably high blood drug levels at each time point. The absolute bioavailability of dry suspensions with RLX and RLX-HICs, calculated according tothe area under the plasma drug concentration-time curve($AUC_{0\text{-}t}$), were3.15% and 13.04, respectively. The relative bioavailability of RLX was enhanced by 313.97%when RLX-HICs was used in the formulation.

The absolute bioavailability from our observation was close to the reported values (Heringa 2003). The relative bioavailability was higher than or approximate to those of literature using solid dispersions (Tran et al. 2013a,b) and solid lipid nanoparticles (Burra et al. 2013; Ravi et al. 2014). However, it was noted that lipid nanocarriers as oral formulation of RLX must be solidified by spray or freeze-drying to prevent RLX expulsion from the carrier materials. In addition, solid formulations containing lipid components are inconvenient in administration because of poor dispersity in water. The potential of cyclodextrin in absorption enhancement of drug has been verified (Liu et al. 2014b). RLX-HICs not onlypossessed high drug dispersity, but also additional advantages, such as improved drug properties and increased GI stability. For those reasons, dry suspensions prepared from drug/cyclodextr in inclusion complexes can serve as an excellent formulation for oral delivery of poorly water-soluble drugs.

2.6. Conclusions

This work demonstrates the suitability and potential of dry suspensions loading RLX-HICs as oral formulation of RLX. Dry suspensions prepared from RLX-HICs significantly enhanced the dissolution and bioavailability of RLX. In addition to biochemical factors, the dissolution of drug, working as a limited factor of drug absorption, largely eclipses the therapeutic efficacy of active pharmaceutical ingredients. Dry suspensions containing RLX-HICs not only improve the dispersibility of drug and hence the dissolution, but also ameliorate the physiochemical attributes of drug owing to complexation. The solid formulation with RLX-HICs is also advantageous in use and storage. Therefore, our study would provide valuable information to develop novel oral dosage forms of poorly water-soluble drugs using a combined formulation strategy.

3. Experimental

3.1. Materials

Raloxifene hydrochloridewas purchased from RunhaoBio-Tech Co., Ltd (Guangzhou, China).HP-β-CDwasobtained from Maxdragon Biochemical Technology Co. Ltd (Guangzhou, China), havinga molar substitution of 0.58∼0.73 and molecular weight of ∼1400. Carboxymethyl starch sodium, hydroxypropyl cellulose, sucrose, and talcwere provided by Shanhe Pharmaceutical Excipients Co., Ltd (Huainan, China). HPLC-grade acetonitrile was supplied by Merck (Darmstadt, Germany). Deionized water was prepared with a water purifying system (Woter, Chengdu, China). All other chemicals or reagents were of analytical grade and used as received.

3.2. Phase solubility study

Phase solubility diagram was drawn according to Higuchi and Connors' method (Higuchi and Connors 1965). In brief, excess RLX was added into 5 mL of deionized water that contains different concentrations of HP-β-CD (10, 30, 40, 50, 60, 70 and 80 mg/mL). Samples were stirred at 600 rpm with a magnetic stirrer for 72 h at 25 °C in a sealed container. Afterwards, the samples were centrifuged (Eppendorf, Hauppauge, USA)at 12,000 g for 5 min. RLX concentration in the supernatants was quantified by the established HPLC method below. The phase solubility diagram was plotted with RLX concentration *versus* HP-β-CD concentration. The complex formation constant (K_f) was calculated based on the equation: $K_f = S/S_0(1\text{-}S)$, where S and S_0 denote the slope of linear equation (if applicable) and solubility of RLX in the absence of HP-β-CD, respectively.

3.3. Preparation of RLX-HICs

RLX-HICs were prepared by thesolvent evaporation technique (Semalty et al. 2014). Typically, RLX and HP-β-CDat a molar ratio of 1: 1.25 were dissolved in 75% ethanoland then the solvent was removed under reduced pressureusing a rotatory evaporator at 45 °C until no residual solvents were remained. The products were harvested by peeling them from the flask.

3.4. Characterization of RLX-HICs

3.4.1. Scanning electron microscopy (SEM)

The morphology of RLX-HICs was inspected by SEM.Briefly, the sample was first smashed using a set of pestle/ mortar. Then, RLX-HICs powders were mounted onto the stubs using double-sided adhesive tape. After the procedure of sputter coating with gold/palladium alloy, the surface of RLX-HICs was scanned and photographed with SEM (Zeiss EVO LS-15, Oberkochen, Germany).

3.4.2. Differential scanning calorimetry (DSC)

DSC measurement was performed on the DSC 204A/G phoenix instrument (Netzsch, Baveria, Germany). Samples of RLX, HP-β-CD, physical mixture(molar ratio of 1:1.25), and RLX-HICs were placed in non-hermetically sealed aluminum pans, and then subjected to the rmalscanning. The samples were heated from 25 to 300 °C at a heating rate of 10 °C/min. The instrument was calibrated with a standard material of indium. All the DSC processes were carried out under nitrogen atmosphere at a flow rate of 100 mL/min.

3.4.3. Fouries transform infrared spectrometry (FTIR)

FTIR spectra were traced to further probe the possible interactions between RLX and HP-β-CD upon complexation. In brief, samples of RLX, HP-β-CD, physical mixture (molar ratio of 1:1.25) and RLX-HICs were ground thoroughly with KBr to obtain infrared transparent matrixes. FTIR measurement was performed on the FTIR-8400S spectrometer (Shimadzu, Toyota, Japan). Spectra were collected from 3500 to 600 cm^{-1} with a resolution of 0.01 cm^{-1}.

3.5. Preparation of dry suspensions

The common wet granulation process was employed to prepare dry suspensions loading RLX-HICs or dry suspensions loading RLX. The compositions of two formulations are listed in Table 3 Firstly, RLX-HICs or RLX plus sucrose (instead of HP-β-CD) was blended with hydroxypropyl cellulose using a shear mixer (Joyoung, Jinan, China), and then passed through a 80-mesh sieve. After that, carboxymethyl starch sodium adsorbing anhydrous

Table 3: Formulation compositions of dry suspensions loading RLX-HICs (DS-RLX-HICs) or dry suspensions loading RLX (DS-RLX)

Components	DS-RLX-HICs	DS-RLX
RLX-HICs	100 mg	/
RLX	/	22.6 mg
Sucrose	/	77.4 mg
Carboxymethyl starch sodium	100 mg	100 mg
Hydroxypropyl cellulose	200 mg	100 mg
Talc	10 mg	10 mg

ethanol (1 g to 0.18 mL) was introduced into the mixer to granulate for 2 min at 1,800 rpm. The wet granules passed a through 40-mesh sieve and were subsequently dried at 45 °C for 2 h. Finally, the resulting dry granules were fully mixed with a specified amount of talc to complete the preparation of dry suspensions.

3.6. Characterization of dry suspensions

The performance of dry suspensions was evaluated by dispersibility, powder flowability, drug content, and related substancesas well. To investigate the dispersibility, 1 g of dry suspensions was added into a 100 mL beaker with 50 mL of water followed by shaking with hand at a frequency of 20 times per minute. If the complete dispersion was within 1, 2 min or over 5 min, the dispersibility performance would be graded as good, medium or weak, respectively. As such, the redispersibility of dry suspensions upon sedimentation was assessed following the similar manner.

Repose angle was adopted to appraise the flow ability of dry suspensions. Briefly, bulk dry suspensions were poured onto a horizontal plate along a funnel, which was hung over the Plate 10 cm, to form a conical pile. The internal angle between the surface of the pile and the horizontal surface was measured for the calculation of repose angle.

The drug content and related substances of three batches of dry suspensions were analyzed by HPLC. For content assay, 100 mg of test samples were dissolved in 100 mL of 75% methanol (v/v) and sonicated for 5 min to extract RLX. After filtration, RLX concentration in filtrates was quantified with standard RLX solution. RLX content was calculated from the weight ratio of RLX to preparations. Self-control law was adopted to determine the general related substances. The test solution, identical to the content assay one, was diluted 100 times, resulting in 1% control solution peak area. The related substances were quantified by comparing the total peak area of impurities with the peak areaof the main component.

3.7. Dissolution experiments

Dissolutions of raw RLX and its preparations were carried out in 900 mL water using ZRS-8G dissolution tester (Tianjin, China), according to Chinese Pharmacopeia (2010) Method II (the paddle method). Samples of RLX and dry suspensions with RLX-HICs or RLX, equilibrant to 50 RLX, were put into the dissolution cup and thermostatically maintained at 37 °Cat a rotation speed of 75 rpm. At predetermined intervals, 5 mL of sample was withdrawn and immediately replenished by the same volume of fresh medium to keep a constant volume. The samples were filtered with Millex® AP membrane (Millipore, 0.45 μm), and RLX concentration in filtrates was determined by HPLC.

3.8. Bioavailability studies

Sprague Dawley rats (200 ± 10 g) were randomly divided into three groups ($n = 5$). Rats were fasted for 24 h before the experiment but had free access to water. The rats were received intragastrically dry suspensions with RLX-HICs or RLX (dispersed in water) at a dose of 30 mg/kg, or intravenously RLX solution (dissolved in 50% PEG 400/water, v/v) at a dose of 5 mg/kg. Blood samples about 0.3 mLwere withdrawn from the tail or jugular veinat specified time points (0.25, 0.5, 1.0, 2.0, 4, 6, 8, 10, 12, and 24 h) after administration. Plasma was separated by centrifugation at 5,000 g for 5 min.All animal experiments were executed according to the Guidelines on the Care and Use of Animals for Scientific Purposes. The protocols for the animal studies were also reviewed and approved by the Experimental Animal Ethical Committee of Shandong University.

Raloxifene in rat plasma was retrieved by a deproteinization procedure with acetonitrile. Typically, three aliquots of acetonitrile added into one aliquot of plasma supplemented with 10 μL of 8 μg/mL fenofibrate as internal standard. Then, the samples were vortexed vigorously for 3 min and centrifuged at 12,000 g for 8 min. The supernatants werecollectedinto centrifuge tubes followed by vacuum evaporation at 35 °C using Concentrator Plus (Eppendorf, Hauppauge, NY, USA). The residues were reconstituted in 100 μL 75% acetonitrile and analyzed by UPLC-QTOF/MS. Non-compartmental analysis was used to process the data and calculate the pharmacokinetic parameters with free *PK* Solver 2.0 software.

3.9. Quantification of RLX

Raloxifene concentration in *in vitro* samples was determined by HPLC (Dionex Ultimate 3000, Thermo Scientific, MA, USA).The HPLC system was equipped with a quaternary pump, a degasser, an autosampler, a column heater, and a multichannel rapid scanning UV-VIS detector. RLX was separated by aDiamonsilC18column (5 μm, 4.6 mm × 250 mm) guarded with a precolumn at 35 °C. The injection volume and detection wavelength were 20 μL and 284 nm, respectively. A mobile phase ofacetonitrile/0.1% phosphoric acid solution (35/65, v/v) was used to elute the analytes with a flow rate of 1.0 mL/min.

Raloxifenein all *in vivo* samples (unless specified otherwise) was quantified by Waters UPLC-QTOF/MS that contains an ACQUITY UPLC system and Xeno G2 QTOF (Milford, MA, USA). Chromatographic elution was performed on an ACQUITY UPLC BEH column (2.1 × 100 mm, 1.7 μm; Waters) using a gradient of 0.1% formic acid in water (mobile phase A) *versus* 0.1% formic acid in acetonitrile (mobile phase B) at a flow rate of 0.45 mL/min. The gradient elution followed the sequence of 5% B at 0 to 1 min, 5 to 95% B at 1 to 3 min, 95% B at 3 to 3.5 min, and 95 to 5% B at 3.5 to 4 min. Quantification was based on the full scan analysis and extracted positive ion chromatograms using MassLynx version 4.1. Configuration and parameter settings of the instrument referred to the reported procedure (Liu et al. 2014a).

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