



Improved dissolution rate and oral bioavailability of lovastatin in red yeast rice products

Chia-Hao Chen^a, Jyh-Chin Yang^b, Yow-Shieng Uang^c, Chun-Jung Lin^{a,*}

^a School of Pharmacy, College of Medicine, National Taiwan University, Taipei 100, Taiwan

^b Department of Internal Medicine, Hospital and College of Medicine, National Taiwan University, Taipei 100, Taiwan

^c School of Pharmacy, Taipei Medical University, Taipei 110, Taiwan

ARTICLE INFO

Article history:

Received 22 November 2012

Accepted 12 January 2013

Available online xxx

Keywords:

Lovastatin
Bioavailability
Dissolution
Crystallinity
Red yeast rice

ABSTRACT

Lovastatin, categorized as a class II compound according to the Biopharmaceutics Classification System, is mainly responsible for the blood cholesterol lowering effect of red yeast rice (RYR). The aim of this study was to compare the dissolution rate, physical state, and oral bioavailability of lovastatin in three RYR products (LipoCol Forte, Cholestin, or Xuezhikang) to those of two lovastatin tablets (Mevacor or Lovasta). The results showed that the dissolution rate of lovastatin in various dissolution media in the registered RYR products was faster and higher than that of lovastatin in lovastatin tablets. Powder X-ray diffraction and differential scanning calorimetry patterns showed that the crystallinity of lovastatin was reduced in RYR products. In human studies, the AUC and C_{\max} values for both lovastatin and its active metabolite, lovastatin acid, were significantly higher in volunteers receiving LipoCol Forte capsules or powder than in those receiving lovastatin tablets or powder. In addition, shorter and less variable T_{\max} values were observed in volunteers taking LipoCol Forte than in those taking lovastatin tablets. These findings suggest that the oral bioavailability of lovastatin is significantly improved in RYR products as a result of a higher dissolution rate and reduced crystallinity.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Cardiovascular disease remains the leading cause of morbidity and mortality worldwide and hyperlipidemia is a major factor contributing to its development (Lewington et al., 2007). Red yeast rice (RYR), a fermented rice product generally produced from a specific strain of red yeast called *Monascus purpureus*, is a popular complementary medicine used to lower blood lipid levels (Heber et al., 1999; Lin et al., 2005; Liu et al., 2006; Huang et al., 2007; Zhao et al., 2007; Lu et al., 2008). The major active component of RYR is considered to be monacolin K, also known as lovastatin, the active ingredient of the statin drug Mevacor (Merck & Co. Inc., USA) (Klimek et al., 2009). Lovastatin is a prodrug that is converted by esterases to its active form, lovastatin acid, which inhibits the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway in the liver (Alberts et al., 1980). In addition to lovastatin, RYR products also contain several monacolins, unsaturated fatty acids, carbohydrate, protein, sterols, amino acids, isoflavones, alkaloid, and trace elements (Heber et al., 1999; Lin et al., 2005; Shang et al., 2012).

Lovastatin, categorized as a class II compound according to the Biopharmaceutics Classification System (BCS) (Wu and Benet, 2005), exhibits poor oral bioavailability (<5%) because of its low solubility (1.3 $\mu\text{g}/\text{mL}$ in water) (Serajuddin et al., 1991), extensive metabolism in the gut and liver (Wang et al., 1991), and transmembrane efflux via P-glycoprotein (P-gp) (Chen et al., 2005). Its bioavailability can be improved by increasing the dissolution rate (Suresh et al., 2007; Patel et al., 2008; Wu et al., 2011) and/or decreasing pre-systemic clearance (Neuvonen et al., 2006, 2008). It was recently demonstrated that extracts of RYR products are more effective in inhibiting CYP450 enzymes and P-gp than pure lovastatin in vitro (Chen et al., 2012). RYR product extracts also show higher lovastatin absorption than pure lovastatin in a model of intestinal absorption using cultured Caco-2 cells (Chen et al., 2012). Thus, the oral bioavailability of lovastatin in RYR products may be different from that of lovastatin in lovastatin tablets.

Several clinical trials have found that 5–6 mg/day of lovastatin in RYR has a comparable efficacy to 20–40 mg/day of pure lovastatin in lowering blood cholesterol (Heber et al., 1999; Becker et al., 2008, 2009) suggesting additive and/or synergistic pharmacological effects of RYR components. However, it is not clear whether, or how, RYR components increase the bioavailability of lovastatin, which, in turn, partly contributes to the cholesterol lowering effects of RYR. In this study, we first investigated the in vitro dissolution of pure lovastatin and lovastatin from lovastatin tablets or registered RYR products (LipoCol Forte, Cholestin, and Xuezhikang).

* Corresponding author at: School of Pharmacy, College of Medicine, National Taiwan University, 1 Jen-Ai Road, Section 1, Taipei 100, Taiwan.
Tel.: +886 2 23123456x88386; fax: +886 2 2391 9098.

E-mail address: clementumich@ntu.edu.tw (C.-J. Lin).

Powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) analyses were also performed to investigate the physical characteristics of different lovastatin preparations. In addition, the systemic exposures of lovastatin and its active metabolite, lovastatin acid, were measured in healthy volunteers taking the RYR product LipoCol Forte capsules or powder or a lovastatin tablet or powder.

2. Materials and methods

2.1. Materials

LipoCol Forte capsules were purchased from NatureWise Biotech & Medicals Corporation, Taiwan, Xuezhikang capsules from Peking University WBL Biotech Co., Ltd., China, and Cholestin capsules from Nu Skin Pharmanex, USA. Pure lovastatin was obtained from Merck KGaA (Darmstadt, Germany). Mevacor (brand name) and generic drug Lovasta lovastatin tablets were purchased, respectively, from Merck, USA and Winston Medical Supply Co., Ltd, Taiwan. Sodium lauryl sulfate (SLS) and sodium taurocholate were purchased from Sigma–Aldrich (St. Louis, MO, USA) and lecithin from Alfa Aesar (Ward Hill, MA, USA). All other reagents and solvents were obtained from standard sources and were of the highest quality available.

2.2. Dissolution experiments

The amounts (mg/capsule) of lovastatin in RYR products, LipoCol Forte, Xuezhikang, and Cholestin, were measured according to the methods described in a previous study (Chen et al., 2012). The dissolution of lovastatin from 20 mg of pure lovastatin powder, one Lovasta tablet (containing 20 mg of lovastatin), one 600 mg Cholestin capsule (equivalent to 1 mg of lovastatin), one 300 mg Xuezhikang capsule (equivalent to 2.5 mg of lovastatin), one 600 mg LipoCol Forte capsule (equivalent to 5.7 mg of lovastatin), four 600 mg LipoCol Forte capsules (equivalent to 22.8 mg of lovastatin), or 2400 mg of powder from four LipoCol Forte capsules (equivalent to 22.8 mg of lovastatin) in 900 mL of pH 5 acetate buffer (Serajuddin et al., 1991) with or without SLS (0.2% (Chattopadhyay and London, 1984) or 2% (USP, 2009)) was measured at 37 °C with a stirrer rotation speed at 100 rpm in a RC806 dissolution tester (Shishin Technology Co., Ltd, Taiwan) using an USP 32 apparatus II (paddle method). In addition, the dissolution of one Lovasta tablet, one Cholestin capsule, one Xuezhikang capsule, and four LipoCol Forte capsules in simulated fasted and fed state intestinal fluids was also measured under the same dissolution conditions. The compositions of the simulated fasted intestinal fluid (29 mM KH_2PO_4 , 5 mM sodium taurocholate, 1.5 mM lecithin, and 220 mM KCl, pH 6.8) and simulated fed intestinal fluid (144 mM acetic acid, 15 mM sodium taurocholate, 4 mM lecithin, and 190 mM KCl, pH 5) were those described in a previous study (Dressman et al., 1998). After 5, 10, 20, 30, 45, and 60 min, 500 μL of dissolution medium was collected and filtered through a 0.2 μm polyethersulfone membrane filter (Pall Corporation, USA). Samples at pH 6.8 were adjusted to pH 5 by the addition of 5 μL of 2 M acetic acid to 500 μL of sample, then all samples were stored at -20°C until analysis, when the lovastatin concentration in the dissolution medium was measured using the HPLC–UV method (Chen et al., 2012).

2.3. Powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC)

Since RYR is composed of about 1% lovastatin and 70% starch (Heber et al., 1999; Lin et al., 2005), 1% lovastatin was prepared by diluting pure lovastatin with starch for comparison for RYR powder, then pure lovastatin, starch, 1% lovastatin in starch, and RYR

product powders (from LipoCol Forte, Xuezhikang, or Cholestin) were subjected to PXRD and DSC analyses. The X-ray diffraction pattern of lovastatin in samples was determined by PXRD (Bruker D2 Phaser Table-top Diffractometer) using Cu-K α radiation at 30 kV and 10 mA, with a step size of 0.04° (2θ) and a step time of 5 s between 5° and 35° (2θ). DSC analysis was performed in a Diamond DSC (Perkin-Elmer Corporation, USA) using 2 mg of pure lovastatin or 10 mg of other samples and a heating rate of 10 °C/min in the range of 60–240 °C in a nitrogen atmosphere.

2.4. Clinical studies

These research studies were approved by the institutional review board and ethics committee before initiation and were registered at ClinicalTrials.gov (NCT01346670 and NCT01527669). All procedures were performed in accordance with the Declaration of Helsinki and according to good clinical practice guidelines and any relevant local laws, regulations, and guidelines. The subjects were 21- to 33-years-old and in good health, as determined by past medical history, physical examination, electrocardiogram, chest X-ray, laboratory tests, and urinalysis. Premenopausal women were required to have a negative pregnancy test. All subjects had to sign an informed consent form before being included in the study. The major exclusion criteria included clinical significant abnormalities detected on physical examination with the potential to alter the absorption, metabolism, or elimination of the study drugs or that could constitute a risk to the subject when taking the study drugs. In addition, subjects were excluded if they had taken any prescription or over-the-counter medications within 14 days before administration of the study drugs.

Two relative bioavailability studies were conducted. In the first (NCT01346670), 14 healthy male subjects were randomly allocated to receive a single dose of either four 600 mg LipoCol Forte capsules (total of 22.8 mg of lovastatin) or one Mevacor tablet (20 mg of lovastatin). In the second (NCT01527669), 12 healthy volunteers (6 male, 6 female) were randomly allocated to receive a single dose of either 2400 mg of powder from four LipoCol Forte capsules (total of 22.8 mg of lovastatin) or the powder from one ground Lovasta tablet (20 mg of lovastatin). The healthy subjects were divided into two groups and a single-dose, two-way crossover study was carried out with a washout period of 7 days. The subjects were fasted for at least 10 h before dosing. The investigational products were administered with 240 mL of water with the subject in an upright position. Blood samples were collected prior to drug administration (T0) and at 0.25 (only in the second study), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after dosing using 10 mL vacutainers containing heparin and sodium fluoride. Within 30 min of collection, the samples were centrifuged at 2600 $\times g$ for 10 min at 4 °C and the plasma transferred to labeled polypropylene tubes containing 50 μL of 17% phosphoric acid and stored at -80°C until analysis. The analytical methods used to measure lovastatin and lovastatin acid in plasma were those described in a previous study (Chen et al., 2012).

2.5. Pharmacokinetic calculations

The plasma concentration–time data were used to calculate the peak concentration in the plasma (C_{max}), the time to reach the peak concentration (T_{max}), and the area under the plasma concentration vs. time curve from time zero to the time of the last quantifiable concentration (AUC_{0-12}) of lovastatin and lovastatin acid by the non-compartment method using WinNonlin™ (version 5.2, Pharsight, USA). The relative bioavailability was estimated as the dose-corrected AUC_{0-12} for the RYR product divided by that for the lovastatin tablet.

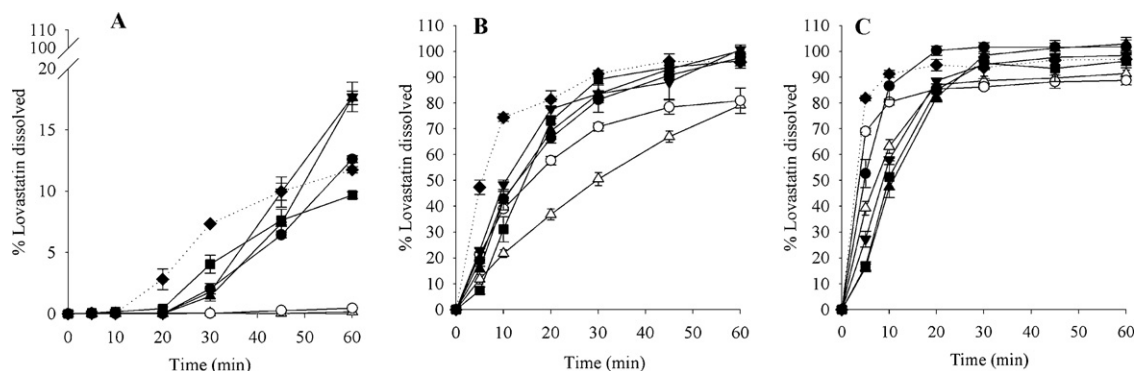


Fig. 1. Dissolution of lovastatin from (Δ) pure lovastatin powder (20 mg), (\circ) one Lovasta tablet (containing 20 mg of lovastatin), (\blacktriangle) one Cholestin capsule (equivalent to 1 mg of lovastatin), (\bullet) one Xuezhikang capsule (equivalent to 2.5 mg of lovastatin), (\blacktriangledown) one LipoCol Forte capsule (equivalent to 5.7 mg of lovastatin), (\blacksquare) four LipoCol Forte capsules (equivalent to 22.8 mg of lovastatin), and (\blacklozenge) 2400 mg LipoCol Forte powder (equivalent to 22.8 mg of lovastatin) in 900 mL of pH 5 acetate buffer alone (A) or containing 0.2% SLS (B) or 2% SLS (C) at 37 °C at a stirrer rotation speed of 100 rpm. The data are the mean \pm SEM ($n = 3$).

2.6. Statistical analysis

Statistical analyses were performed using SYSTAT v12 (SYSTAT Software, Inc., Chicago, IL, USA). Statistical differences were evaluated by the analysis of variance with a level of significance of 0.05. Pairwise comparisons between groups were made using Fisher's least-significant difference test.

3. Results

3.1. Dissolution in acetate buffer

Since it has been demonstrated that lovastatin is more stable at pH 5, but its solubility is not pH-dependent (Serajuddin et al., 1991), the dissolution of lovastatin was first investigated in acetate buffer at pH 5. As shown in Fig. 1A, almost no lovastatin was dissolved from pure lovastatin powder or a lovastatin tablet, whereas the percentage of lovastatin dissolved was significantly higher for all RYR products. These data suggest that the dissolution of lovastatin is improved by other RYR components. Although lovastatin was practically insoluble in acetate buffer, the addition of 0.2% (Fig. 1B) or 2% (Fig. 1C) SLS significantly improved its dissolution in a dose-dependent manner and also increased lovastatin release from all RYR products.

3.2. Dissolution in simulated fasted and fed state intestinal fluid

Since lovastatin is a BCS class II drug, i.e. it has low aqueous solubility and high intestinal permeability, the ingredients of the dissolution medium play an important role in its dissolution, as shown in Fig. 1B and C. However, since it has been reported that SLS is not an adequate surrogate for bile components in the dissolution medium (Shah et al., 1989; Galia et al., 1998), simulated fasted and fed state intestinal fluids were then used to represent the conditions in the intestinal medium. As with the studies in Fig. 1, the dissolution of lovastatin from both lovastatin tablets and RYR products was significantly improved in both the simulated fasted (Fig. 2A) and fed (Fig. 2B) intestinal fluids and the RYR products showed a faster and higher dissolution rate than lovastatin tablets. It was also noted that the percentage of lovastatin released from lovastatin tablets was about 2-fold higher in the fed state than in the fasted state intestinal fluid, whereas no significant difference was seen in dissolution rate of RYR products in the two simulated media. These data indicate that ingestion of food has a greater effect on the dissolution of lovastatin tablets than that of RYR products.

3.3. Physical characterization of lovastatin

Given that the dissolution/solubility of lovastatin was significantly higher for RYR products than lovastatin tablet/powder

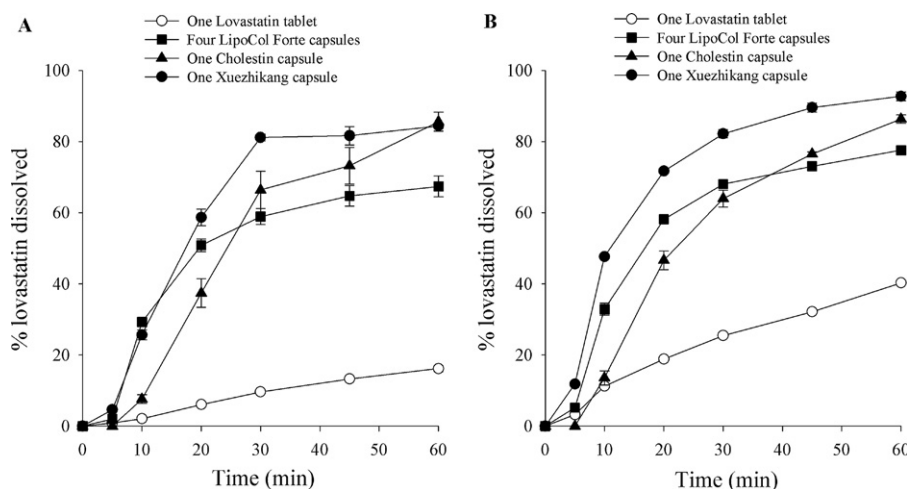


Fig. 2. Dissolution of lovastatin from one Lovasta tablet (20 mg of lovastatin), four LipoCol Forte capsules (equivalent to 22.8 mg of lovastatin), one Cholestin capsule (equivalent to 1 mg of lovastatin), and one Xuezhikang capsule (equivalent to 2.5 mg of lovastatin) in 900 mL of simulated fasted intestinal fluid (A) or simulated fed intestinal fluid (B) at 37 °C with a stirrer rotation speed of 100 rpm. The data are the mean \pm SEM ($n = 3$).

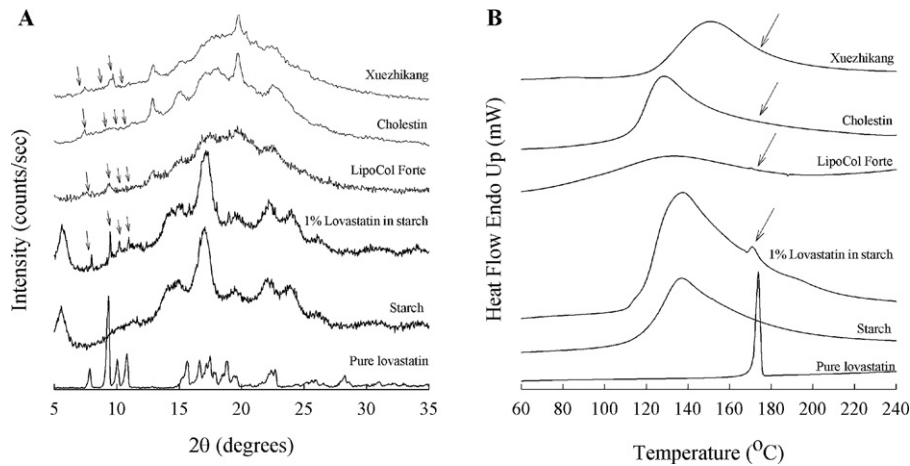


Fig. 3. PXRD (A) and DSC (B) patterns for pure lovastatin, starch, 1% lovastatin in starch, and the RYR products, LipoCol Forte, Cholestin, and Xuezhikang.

under different conditions, PXRD and DSC were used to determine whether the physical characteristics of lovastatin were different in RYR products. As shown in Fig. 3A, the X-ray diffraction pattern of pure lovastatin had sharp peaks at diffraction angles (2θ) of 7.85° , 9.35° , 10.04° , and 10.81° , similar to previous findings (Suresh et al., 2007; Patel et al., 2008; Wu et al., 2011). The same sharp peaks were also observed in the PXRD pattern of 1% lovastatin in starch, indicating that the crystal form of lovastatin was preserved when lovastatin powder was diluted with starch.

However, at the same lovastatin level (i.e., 1%), the intensity of these peaks was lower in the RYR powders (LipoCol Forte, Cholestin, and Xuezhikang), indicating that the crystallinity of lovastatin was significantly decreased in RYR products. In the DSC analysis (Fig. 3B), the pure lovastatin exhibited a sharp melting peak at about 173°C , consistent with a previous finding (Patel et al., 2008), and the thermogram for 1% lovastatin in starch showed a small broad melting peak at about 170°C . However, powders from RYR products (LipoCol Forte, Cholestin, and Xuezhikang) did not show this

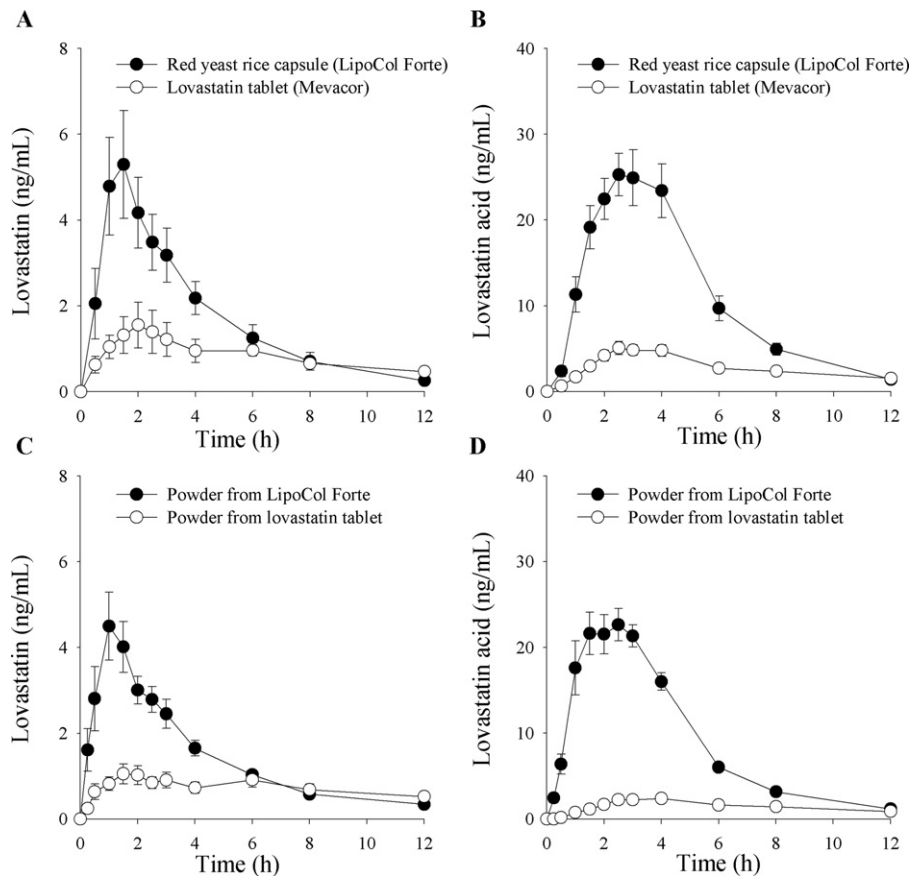


Fig. 4. Plasma concentrations of lovastatin and lovastatin acid in healthy subjects after administration of a single dose of RYR product or lovastatin tablet in the fasted state. The upper panels (A and B) show the results of taking a single dose of four 600 mg LipoCol Forte capsules (22.8 mg of lovastatin) or one Mevacor tablet (20 mg of lovastatin) ($n=14$). The lower panels (C and D) show that results of taking 2400 mg of powder from four LipoCol Forte capsules (22.8 mg of lovastatin) or powder from one ground Lovasta tablet (20 mg of lovastatin) ($n=12$). The data are the mean \pm SEM.

Table 1
Pharmacokinetic parameters for lovastatin and lovastatin acid in healthy subjects after administration of a single dose of four 600 mg LipoCol Forte capsules (22.8 mg of lovastatin) or one Mevacor tablet (20 mg of lovastatin) in the fasted state. The data are the mean ±SD for 14 subjects.

	Lovastatin		Lovastatin acid	
	LipoCol Forte capsules	Mevacor Tablet	LipoCol Forte capsules	Mevacor tablet
AUC ₀₋₁₂ (ng h/mL)	20.6 ± 14.9 ^{***}	10.1 ± 6.8	131.0 ± 43.9 ^{***}	33.7 ± 12.3
C _{max} (ng/mL)	6.64 ± 4.68 ^{***}	1.90 ± 1.90	30.50 ± 11.39 ^{***}	5.92 ± 2.77
T _{max} (h) (range)	1.68 ± 0.99 [*] (0.5–4)	3.75 ± 3.15 (0.5–12)	2.54 ± 0.80 ^{**} (1.5–4)	3.54 ± 0.97 (2–6)
Bioavailability (%)	181 ± 46	100	352 ± 85	100

^{*} p value <0.05 versus the Mevacor tablet group.
^{**} p value <0.01 versus the Mevacor tablet group.
^{***} p value <0.001 versus the Mevacor tablet group.

typical melting peak of lovastatin at about 170 °C. The PXRD and DSC results both suggest that the crystallinity of lovastatin is significantly reduced in RYR products.

3.4. Clinical pharmacokinetic studies of LipoCol Forte

Given the similarity of the PXRD and DSC findings and the dissolution profiles of lovastatin in the tested RYR products, LipoCol Forte was chosen for two clinical pharmacokinetic studies in healthy volunteers. Plasma concentrations of lovastatin and its active metabolite, lovastatin acid, were measured to examine the in vivo effects of RYR components on lovastatin bioavailability.

In the first study comparing a single dose of either four LipoCol Forte capsules (22.8 mg of lovastatin) and one Mevacor tablet

(20 mg of lovastatin) in 14 volunteers, the results showed that plasma levels of lovastatin (Fig. 4A) and lovastatin acid (Fig. 4B) were much higher in subjects given the LipoCol Forte capsules. The AUC and C_{max} values for both lovastatin and lovastatin acid were also significantly higher in the volunteers who received the LipoCol Forte capsules than in those who received Mevacor (Table 1). Shorter and less variable T_{max} values were also observed in the volunteers taking LopoCol Forte capsules (Fig. 5A and B). These findings demonstrate that the absorption rate and systemic exposure of lovastatin are both increased in RYR products.

Since dissolution is the rate-limiting step for the oral absorption of BCS class II drugs, such as lovastatin, the increase in lovastatin bioavailability in RYR could be due to formulation or ingredients. To reduce the effect of disintegration, 2400 mg of powder from four

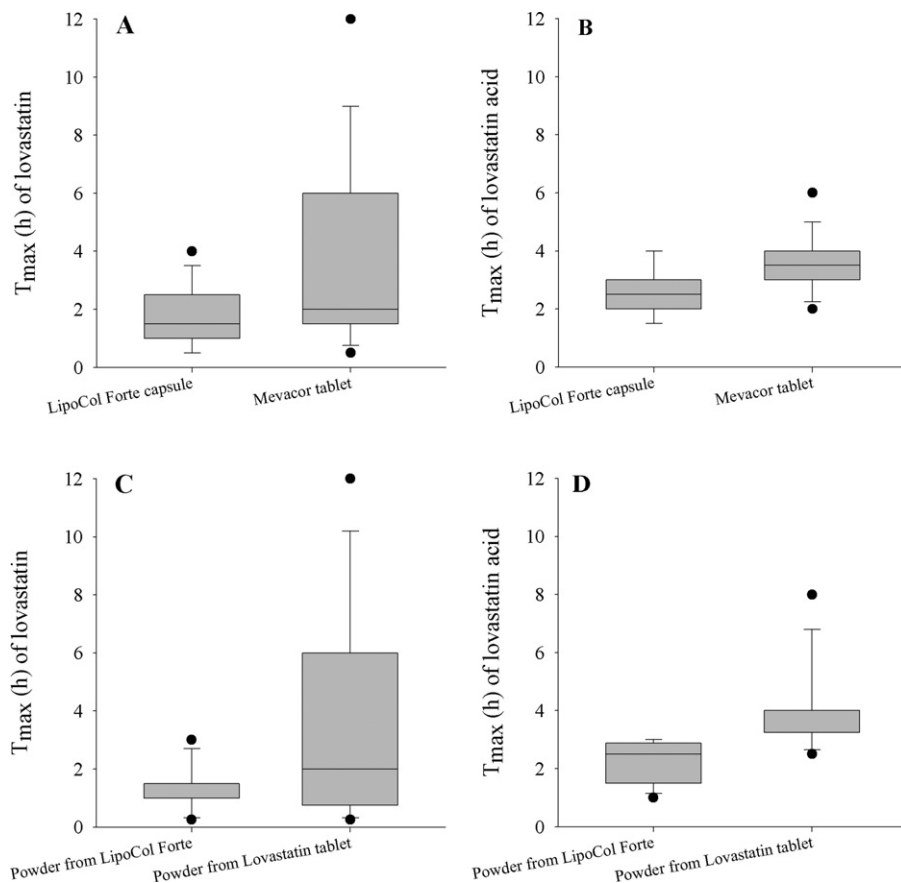


Fig. 5. Distribution of T_{max} values for lovastatin and lovastatin acid in healthy subjects after administration of a single dose of RYR product or lovastatin tablet in the fasted state. Panels A and B show the results of taking four 600 mg LipoCol Forte capsules or powder from one Lovasta tablet, while panels C and D show the results of taking 2400 mg of powder from four LipoCol Forte capsules or powder from one Lovasta tablet. The boxes represent the median and interquartile range, the bars represent the 10th percentile and 90th percentile, and the circles depict outliers at a distance greater than 1.5 times the box height.

Table 2

Pharmacokinetic parameters for lovastatin and lovastatin acid in healthy subjects after administration of a single dose of 2400 mg of powder from four LipoCol Forte capsules (22.8 mg of lovastatin) or powder from one Lovasta tablet (20 mg of lovastatin) in the fasted state. The data are the mean \pm SD for 12 subjects.

	Lovastatin		Lovastatin acid	
	LipoCol Forte powder	Lovastatin tablet powder	LipoCol Forte powder	Lovastatin tablet powder
AUC _{0–12} (ng h/mL)	17.4 \pm 6.2 ^{***}	8.9 \pm 4.9	108.6 \pm 21.7 ^{***}	17.4 \pm 8.0
C _{max} (ng/mL)	4.99 \pm 2.82 ^{***}	1.32 \pm 0.79	26.22 \pm 8.23 ^{***}	2.80 \pm 1.66
T _{max} (h) (range)	1.23 \pm 0.72 (0.25–3)	3.40 \pm 3.48 (0.25–12)	2.21 \pm 0.69 ^{***} (1–3)	4.04 \pm 1.36 (2.5–8)
Bioavailability (%)	202 \pm 78	100	646 \pm 288	100

* *p* value < 0.05 versus the lovastatin tablet group.

*** *p* value < 0.001 versus the lovastatin tablet group.

LipoCol Forte capsules (22.8 mg of lovastatin) and the powder from one ground Lovasta tablet (20 mg of lovastatin) were used in a second pharmacokinetic study in 12 volunteers. The results showed that the plasma concentration, C_{max}, and AUC for both lovastatin and lovastatin acid were again significantly higher in the subjects taking LipoCol Forte powder than in those taking the Lovasta tablet powder (Fig. 4C and D, Table 2). In addition, similar to the results in the previous study, the T_{max} values for lovastatin and lovastatin acid were smaller and less variable in the subjects taking LipoCol Forte powder than in those taking the Lovasta tablet powder (Fig. 5C and D). These results suggest that the formulation (LipoCol Forte capsule vs. lovastatin tablet) is not an important factor in the altered pharmacokinetic properties of lovastatin and lovastatin acid seen in RYR products compared to lovastatin tablet.

4. Discussion

Lovastatin is the major active component of RYR products, and several clinical trials have shown that 5–6 mg/day of lovastatin given as an RYR product has a comparable efficacy to 20–40 mg/day of lovastatin tablet in lowering blood cholesterol (Heber et al., 1999; Becker et al., 2008, 2009). Although these findings may be due to additive and/or synergistic pharmacological effects of RYR components, the present study demonstrated that the oral bioavailability of lovastatin was significantly increased when it was given as an RYR product. The increase in the systemic exposure of lovastatin and its active metabolite, lovastatin acid, may then contribute to the greater potency of RYR products in lowering cholesterol. In addition, the variation in the absorption rate (reflected by T_{max}) of lovastatin was also minimized by giving RYR products.

Lovastatin is categorized as a BCS class II drug (i.e., high permeability, but low solubility) and its dissolution rate is expected to limit its absorption from the gastrointestinal tract. Thus, increasing the dissolution rate and/or decreasing the pre-systemic clearance might enhance the oral bioavailability of lovastatin. We found that the release of lovastatin from tablets was highly dependent on the dissolution conditions, the percentage release being 160-fold and 180-fold higher in the presence of 0.2% SLS and 2% SLS, respectively, than in acetate buffer, suggesting an important role of the surfactant in lovastatin release. Likewise, lovastatin release was also increased using simulated intestinal fluid containing taurocholate and lecithin. These findings are in line with the indication that lovastatin tablets should be given with meals, as the bioavailability of lovastatin increases by 30–50% when taken with a standard meal (Schmidt and Dalhoff, 2002; Package insert of Mevacor, 2012). On the other hand, the dependence of lovastatin release on the dissolution conditions might also lead to a highly variable absorption rate in vivo, as shown by the T_{max} values for lovastatin (0.5–12 h) and lovastatin acid (2–6 h) when lovastatin tablets were taken by healthy volunteers (Fig. 5A and B, Table 1).

In contrast to the medium-dependent release of lovastatin from lovastatin tablets, the dissolution of lovastatin from RYR products was higher and less affected by the dissolution medium. One of the

reasons for this is probably the content of fatty acids and sterols in RYR products (Heber et al., 1999; Lin et al., 2005; Shang et al., 2012), which may facilitate lovastatin dissolution and reduce the impact of the food effect. Also, as shown by the PXRD and DSC analyses, the reduced crystallinity of lovastatin in RYR products may partly contribute to the higher dissolution rate. These improvements in the dissolution of lovastatin may then lead to the faster absorption rate (shorter T_{max}) and the lower intersubject variation in absorption seen with RYR products (Fig. 5). On the other hand, it has been demonstrated that the T_{max} and AUC_{0–12} values for lovastatin are about 3.25 h and 25 ng h/mL, respectively, in healthy subjects taking four LipoCol Forte capsules after high-fat meals (Chen et al., 2012). Comparing the results of the present study conducted in the fasted state and those of our previous study conducted in the fed state (Chen et al., 2012), food consumption did not alter the AUC for lovastatin (25 ng h/mL in the fed state vs. 21 ng h/mL in the fasted state), whereas the T_{max} was increased by food (3.25 h in the fed state vs. 1.68 h in the fasted state). In this regard, the increased dissolution rate seen with RYR products might enable lovastatin (a BCS class II drug) to function as a BCS class I drug, and, if so, high-fat meals will probably not have a significant effect on its bioavailability, but delay stomach emptying and therefore cause an increase in peak time (Wu and Benet, 2005).

In addition to the effects on lovastatin dissolution, other RYR ingredients might also decrease the pre-systemic clearance of lovastatin. In our previous study (Chen et al., 2012), extracts of RYR products (LipoCol Forte, Cholestin, and Xuezhikang) were more effective than pure lovastatin in inhibiting the activities of CYP450 enzymes and P-gp, and also showed higher absorption at the same concentration of lovastatin than pure lovastatin in cultured Caco-2 cells. However, despite the observation that RYR product extracts can inhibit CYP450 enzymes and P-gp in vitro, the pharmacokinetic properties of lovastatin and lovastatin acid in an RYR product (LipoCol Forte) were found to be linear within the usual dose range and no significant accumulation was observed after multiple dosing because the concentration in vivo is too low to have inhibitory effects on CYP450 enzymes and P-gp (Chen et al., 2012). In this regard, the enhanced dissolution rate seen with RYR products is probably the major factor responsible for the increase in absorption and systemic exposure of lovastatin in vivo.

There is little information on the pharmacokinetic properties of lovastatin in RYR products. In the present study, the systemic exposure of lovastatin was much higher when it was given as an RYR product than as lovastatin drug. However, these pharmacokinetic results are not consistent with those of Li et al. who found significantly lower serum lovastatin levels using an RYR product compared to an equivalent cholesterol-lowering dose of lovastatin drug (Li et al., 2005). However, it should be noted that the RYR product and lovastatin in this previous study were taken with 200 mL of double-strength grapefruit juice, a well-known CYP3A4 inhibitor, which can significantly increase the C_{max} and AUC of lovastatin (Kantola et al., 1998), and the results may not truly reflect the pharmacokinetic properties of lovastatin in RYR products.

5. Conclusions

Lovastatin is a BCS class II drug with low solubility and low dissolution rate. However, in RYR products, the dissolution rate and bioavailability of lovastatin is significantly enhanced. The improved dissolution rate in RYR might enable lovastatin to function as a BCS class I drug.

Conflict of interest/disclosure

There is no conflict of interest.

Acknowledgements

This study was supported, in part, by Grant NSC 99-3114-B-002-001 from the National Science Council of Taiwan.

We thank Dr. Ya-Ching Yang from Bruker Taiwan Co., Ltd. for helping with the PXRD assay.

References

- Alberts, A.W., Chen, J., Kuron, G., Hunt, V., Huff, J., Hoffman, C., Rothrock, J., Lopez, M., Joshua, H., Harris, E., Patchett, A., Monaghan, R., Currie, S., Stapley, E., Albers-Schonberg, G., Hensens, O., Hirshfield, J., Hoogsteen, K., Liesch, J., Springer, J., 1980. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proc. Natl. Acad. Sci. U. S. A.* 77, 3957–3961.
- Becker, D.J., Gordon, R.Y., Halbert, S.C., French, B., Morris, P.B., Rader, D.J., 2009. Red yeast rice for dyslipidemia in statin-intolerant patients: a randomized trial. *Ann. Intern. Med.* 150, 830–839.
- Becker, D.J., Gordon, R.Y., Morris, P.B., Yorko, J., Gordon, Y.J., Li, M., Iqbal, N., 2008. Simvastatin vs therapeutic lifestyle changes and supplements: randomized primary prevention trial. *Mayo Clin. Proc.* 83, 758–764.
- Chattopadhyay, A., London, E., 1984. Fluorimetric determination of critical micelle concentration avoiding interference from detergent charge. *Anal. Biochem.* 139, 408–412.
- Chen, C., Mireles, R.J., Campbell, S.D., Lin, J., Mills, J.B., Xu, J.J., Smolarek, T.A., 2005. Differential interaction of 3-hydroxy-3-methylglutaryl-coa reductase inhibitors with ABCB1, ABCC2, and OATP1B1. *Drug Metab. Dispos.* 33, 537–546.
- Chen, C.H., Uang, Y.S., Wang, S.T., Yang, J.C., Lin, C.J., 2012. Interaction between red yeast rice and CYP450 enzymes/P-glycoprotein and its implication for the clinical pharmacokinetics of lovastatin. *Evid. Based Complement. Alternat. Med.*, 10, Article ID 127043.
- Dressman, J.B., Amidon, G.L., Reppas, C., Shah, V.P., 1998. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. *Pharm. Res.* 15, 11–22.
- Galia, E., Nicolaides, E., Hörter, D., Löbenberg, R., Reppas, C., Dressman, J.B., 1998. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. *Pharm. Res.* 15, 698–705.
- Heber, D., Yip, I., Ashley, J.M., Elashoff, D.A., Elashoff, R.M., Go, V.L., 1999. Cholesterol-lowering effects of a proprietary Chinese red-yeast-rice dietary supplement. *Am. J. Clin. Nutr.* 69, 231–236.
- Huang, C.F., Li, T.C., Lin, C.C., Liu, C.S., Shih, H.C., Lai, M.M., 2007. Efficacy of *Monascus purpureus* Went rice on lowering lipid ratios in hypercholesterolemic patients. *Eur. J. Cardiovasc. Prev. Rehabil.* 14, 438–440.
- Kantola, T., Kivistö, K.T., Neuvonen, P.J., 1998. Grapefruit juice greatly increases serum concentrations of lovastatin and lovastatin acid. *Clin. Pharmacol. Ther.* 63, 397–402.
- Klimek, M., Wang, S., Ogunkanmi, A., 2009. Safety and efficacy of red yeast rice (*Monascus purpureus*) as an alternative therapy for hyperlipidemia. *P. T.* 34, 313–327.
- Lewington, S., Whitlock, G., Clarke, R., Sherliker, P., Emberson, J., Halsey, J., Qizilbash, N., Peto, R., Collins, R., 2007. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet* 370, 1829–1839.
- Li, Z., Seeram, N.P., Lee, R., Thames, G., Minutti, C., Wang, H.J., Heber, D., 2005. Plasma clearance of lovastatin versus Chinese red yeast rice in healthy volunteers. *J. Altern. Complement. Med.* 11, 1031–1038.
- Lin, C.C., Li, T.C., Lai, M.M., 2005. Efficacy and safety of *Monascus purpureus* Went rice in subjects with hyperlipidemia. *Eur. J. Endocrinol.* 153, 679–686.
- Liu, J., Zhang, J., Shi, Y., Grimsgaard, S., Alraek, T., Fønnebo, V., 2006. Chinese red yeast rice (*Monascus purpureus*) for primary hyperlipidemia: a meta-analysis of randomized controlled trials. *Chin. Med.* 1, article 4.
- Lu, Z., Kou, W., Du, B., Wu, Y., Zhao, S., Brusco, O.A., Morgan, J.M., Capuzzi, D.M., Li, S., 2008. Effect of xuezhikang, an extract from red yeast Chinese rice, on coronary events in a Chinese population with previous myocardial infarction. *Am. J. Cardiol.* 101, 1689–1693.
- Neuvonen, P.J., Backman, J.T., Niemi, M., 2008. Pharmacokinetic comparison of the potential over-the-counter statins simvastatin, lovastatin, fluvastatin and pravastatin. *Clin. Pharmacokinet.* 47, 463–474.
- Neuvonen, P.J., Niemi, M., Backman, J.T., 2006. Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. *Clin. Pharmacol. Ther.* 80, 565–581.
2012. Package insert of Mevacor. Merck & Co., Inc., Whitehouse Station, NJ 08889, USA http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/019643s085lbl.pdf
- Patel, M., Tekade, A., Gattani, S., Surana, S., 2008. Solubility enhancement of lovastatin by modified locust bean gum using solid dispersion techniques. *AAPS PharmSciTech.* 9, 1262–1269.
- Schmidt, L.E., Dalhoff, K., 2002. Food–drug interactions. *Drugs* 62, 1481–1502.
- Serajuddin, A.T., Ranadive, S.A., Mahoney, E.M., 1991. Relative lipophilicities, solubilities, and structure-pharmacological considerations of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors pravastatin, lovastatin, mevastatin, and simvastatin. *J. Pharm. Sci.* 80, 830–834.
- Shah, V.P., Konecny, J.J., Everett, R.L., McCullough, B., Noorizadeh, A.C., Skelly, J.P., 1989. In vitro dissolution profile of water-insoluble drug dosage forms in the presence of surfactants. *Pharm. Res.* 6, 612–618.
- Shang, Q., Liu, Z., Chen, K., Xu, H., Liu, J., 2012. A systematic review of xuezhikang, an extract from red yeast rice, for coronary heart disease complicated by dyslipidemia. *Evid. Based Complement. Alternat. Med.*, 18, Article ID 636547.
- Suresh, G., Manjunath, K., Venkateswarlu, V., Satyanarayana, V., 2007. Preparation, characterization, and in vitro and in vivo evaluation of lovastatin solid lipid nanoparticles. *AAPS PharmSciTech.* 8, E162–E170.
- USP, 2009. Dissolution test for lovastatin tablet. In: United States Pharmacopeia National Formulary, USP32. United States Pharmacopeial Convention, pp. 2816–2817.
- Wang, R.W., Kari, P.H., Lu, A.Y., Thomas, P.E., Guengerich, F.P., Vyas, K.P., 1991. Biotransformation of lovastatin. IV. Identification of cytochrome P450 3A proteins as the major enzymes responsible for the oxidative metabolism of lovastatin in rat and human liver microsomes. *Arch. Biochem. Biophys.* 290, 355–361.
- Wu, C., Wang, Z., Zhi, Z., Jiang, T., Zhang, J., Wang, S., 2011. Development of biodegradable porous starch foam for improving oral delivery of poorly water soluble drugs. *Int. J. Pharm.* 403, 162–169.
- Wu, C.Y., Benet, L.Z., 2005. Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm. Res.* 22, 11–23.
- Zhao, S.P., Lu, Z.L., Du, B.M., Chen, Z., Wu, Y.F., Yu, X.H., Zhao, Y.C., Liu, L., Ye, H.J., Wu, Z.H., 2007. Xuezhikang, an extract of cholestin, reduces cardiovascular events in type 2 diabetes patients with coronary heart disease: subgroup analysis of patients with type 2 diabetes from China Coronary Secondary Prevention study (CCSPS). *J. Cardiovasc. Pharmacol.* 49, 81–84.