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Enhanced dissolution and bioavailability of biochanin A via the preparation of solid dispersion: In vitro and in vivo evaluation

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ABSTRACT

The present study aimed to improve the bioavailability of biochanin A, a poorly soluble bioflavonoid, via the preparation of solid dispersion (SD) using Solutol® HS15 and HPMC 2910. Solubility of biochanin A was enhanced by 8–60 folds as the drug-carrier ratio was increased in SDs. Furthermore, compared to pure biochanin A or physical mixture (PM), SDs significantly improved the dissolution rate and the extent of drug release. Particularly, SDs (Drug:Solutol® HS15:HPMC 2910 = 1:5:5 or 1:10:10) achieved the rapid and complete drug release (approximately 100% within 1 h) at pH 6.8. The XRD patterns indicated that SDs might enhance the solubility of biochanin A by changing the drug crystallinity to amorphous state in addition to the solubilizing effect of hydrophilic carriers. The improved dissolution of biochanin A via SD formulation appeared to be well correlated with the enhanced oral exposure of biochanin A in rats. After an oral administration of SD (Drug:Solutol® HS15:HPMC 2910 = 1:10:10), C_{max} and AUC of biochanin A were increased by approximately 13 and 5 folds, respectively, implying that SDs could be effective to improve the bioavailability of biochanin A. In conclusion, solid dispersion with Solutol® HS15 and HPMC 2910 appeared to be promising to improve the dissolution and oral exposure of biochanin A.

1. Introduction

Biochanin A (5,7-dihydroxy-4′-methoxy-isoflavone) is one of the major isoflavones found in red clover and certain herbal products, which are marketed for the treatment of post-menopausal symptoms including hot flashes and osteoporosis (Atkinson et al., 2004; Booth et al., 2006; Coon et al., 2007; Risbridger et al., 2001). In addition, biochanin A has various biological activities such as antioxidant, anti-inflammatory, antiviral and anti-carcinogenic effects (Puli et al., 2006). Biochanin A is also known as an inhibitor of P-glycoprotein (P-gp), a major efflux transporter protein (Zhang and Morris, 2003). As P-gp is widely expressed in intestine, liver, blood–brain barrier and kidney, it has a great impact on the absorption, distribution and elimination of various therapeutic compounds including taxol, vinca alkaloids and anthracyclines (Ambudkar et al., 1999; Germann, 1996). Therefore, biochanin A has gained great attention as a potential absorption enhancer for P-gp substrates, based on its strong inhibition effect on P-gp activity in the cell culture systems. However, in contrast to the in vitro results, the oral administration of biochanin A did not improve the bioavailability of P-gp substrates such as doxorubicin, cyclosporine A and paclitaxel in rats even at the high dose (250 mg/kg) (Zhang et al., 2010). This discrepancy between in vitro and in vivo results could be explained by the poor bioavailability of biochanin A. The oral bioavailability of biochanin A is very low (1–2%) that might be related to, at least in part, its low aqueous solubility (Moon et al., 2006). Therefore, improving the solubility and bioavailability of biochanin A should be critical to maximize its utility as a P-gp inhibitor.

Solid dispersion with hydrophilic carriers has been demonstrated as a promising technique for improving the solubility and dissolution rate of poorly water soluble drugs (Vasconcelos et al., 2007). For example, gelucire-based solid dispersion of ritonavir markedly increased the solubility and dissolution of ritonavir, resulting in the enhanced oral exposure of ritonavir (Sinha et al., 2010). Also, the solid dispersion formulation of ibuprofen using poloxamer 407 was effective to improve the dissolution and oral bioavailability of ibuprofen (Newa et al., 2008). Rajebahadur et al. (2006) also have reported that the solubility and dissolution rate of nifedipine were significantly enhanced by the solid dispersion preparation with Solutol® HS15. The improved solubility and dissolution by using solid dispersion formulation could be explained by the particle size reduction, the change of drug crystallinity to amorphous form, the solubilizing effect of hydrophilic carriers and better wettability of drugs surrounded by carriers (Ahuja et al., 2007; Vasconcelos et al., 2007). Therefore, the present study

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aimed to (i) investigate the effect of various hydrophilic carriers on the solubility of biochanin A and (ii) develop the effective solid dispersion formulation improving the dissolution and oral exposure of biochanin A. SD formulations were prepared by using the solvent method at various drug–carrier ratios and their dissolution profiles were evaluated in comparison with the untreated powder and physical mixture (PM). In vitro and in vivo correlation was also examined in rats.

2. Materials and methods

2.1. Materials

Biochanin A and 6-methoxyflavone (internal standard) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Polyethylene glycol 660 hydroxystearate (Solutol® HS15), povidone K-30 (Kollidon® 30), polyethylene glycol 3400 (PEG 3400) and poloxamer 407 (Lutrol® F 127) were obtained from BASF (Ludwigshafen, Germany). Hydroxypropylmethylcellulose (HPMC 2910) was obtained from Whawon Pharm Co. (Seoul, Korea). All other chemicals were of reagent grade and all solvents were of HPLC grade.

2.2. Methods

2.2.1. Carrier screening

The effect of various carriers including povidone K-30, poloxamer 407, HPMC 2910, mannitol, polyethylene glycol 3400 and Solutol® HS15 on the solubility of biochanin A was examined for the preparation of solid dispersion (SD) with biochanin A. First, SDs of biochanin A with each carrier at the drug–carrier ratio of 1:5 was prepared by using the solvent method and then the aqueous solubility of SDs was evaluated as described in Section 2.2.3.1.

2.2.2. Preparation of physical mixtures and solid dispersions

Physical mixtures (PMs) were obtained by mixing biochanin A, HPMC 2910 and Solutol® HS15 using spatula and pestle in a mortar. Solid dispersions (SDs) were prepared by using the solvent method. Briefly, biochanin A and HPMC 2910 were dissolved in 1:1 mixture of ethanol and dichloromethane. Then, Solutol® HS15 dissolved in ethanol was added to the solution and after vigorous mixing, all the solvents were removed under vacuum at room temperature. The weight ratios of drug to each carrier were 1:1:1, 1:3:3, 1:5:5, 1:10:10 and 1:20:20 for PMs and SDs.

2.2.3. In vitro characterizations

2.2.3.1. Solubility tests. Sample amount equivalent to 1 mg of biochanin A was added into 1 ml of distilled water and stirred at 700 rpm for 48 h at room temperature. After centrifuged at 13,000 rpm for 10 min, the supernatant was filtered through 0.2 μm pore-sized cellulose syringe filter (Target®, National scientific, USA) and the drug concentration in the filtrate was measured by using HPLC assay.

2.2.3.2. X-ray diffraction (XRD). X-ray powder diffraction was performed at room temperature with an X-ray diffractometer (X'Pert PRO MPD, PANalytical Co., Holland). Monochromatic Cu Kα-radiation (λ = 1.5418 Å) was obtained with a Ni-filtration and a system of diverging and receiving slides of 0.5° and 0.1 mm, respectively. The diffraction pattern was measured with a voltage of 40 kV and a current of 30 mA over a 2θ range of 3–40° using a step size of 0.02° at a scan speed of 1 s/step.

2.2.3.3. Dissolution studies. Dissolution tests were conducted using the USP paddle method with 50 rpm at 37 ± 0.5 °C in a DST 600A dissolution tester (Fine Science Institute, Korea). The drug release from SDs was evaluated at various drug–carrier ratios in pH 6.8 phosphate buffer and compared to those from pure biochanin A and PMs. In addition, pH-dependency in the dissolution of SDs was examined at pH 1.2, 4.0, and 6.8. Briefly, each formulation was exposed to the dissolution medium (pH 1.2, 4.0, 6.8 buffer and water) for 6 h. At the predetermined time points (5, 10, 15, 30, 45, 60, 120, 240, 360 min), 1 ml of each sample was collected and filtered through 0.45 μm pore-sized PTFE syringe filter. After the each sample collection, an equivalent amount of fresh medium was added to maintain the constant volume of dissolution media. Released drug amount was determined by HPLC assay.

2.2.3.4. Stability test. To evaluate the stability of SD formulations, each sample was placed in the airtight vials and stored at 4 °C or 25 °C. Then, the aqueous solubility as well as XRD patterns of each sample was examined periodically.

2.2.4. HPLC assay

2.2.4.1. In vitro samples. Biochanin A concentration was determined by the HPLC assay as reported by Krenn et al. (2002) with slight modification. The HPLC system consisted of a UV detector (SPD-10A), an automatic injector (SIL-10A), and pumps (LC-10AD). An octadecylsilane column (Gemini C18, 4.6 × 150 mm, 5 μm; Phenomenex, Torrance, CA, USA) was eluted with a mobile phase consisted of water (adjusted with sulfuric acid to pH 2.7): acetonitrile (58:42, v/v%). The flow rate was 1.0 ml/min with the UV detection wavelength set at 254 nm. The retention time of biochanin A and the internal standard (6-methoxyflavone) was 15.5 min and 17.5 min, respectively. The calibration curve of biochanin A was linear within the concentration range of 10–10,000 ng/ml and the limit of detection was 10 ng/ml.

2.2.4.2. Plasma samples. Biochanin A concentration in plasma samples was determined by the HPLC assay as reported by Moon and Morris (2007) with slight modification. Briefly, 10 μl of 6-methoxyflavone (25 μg/ml) as an internal standard was added to each plasma sample (50 μl), and then 190 μl of acetonitrile was added to the mixture. After vortexing for 3 min, the mixture was centrifuged at 13,000 rpm for 10 min and 50 μl of the supernatant was injected into the HPLC system. An octadecylsilane column (Gemini C18, 4.6 × 250 mm, 5 μm; Phenomenex, Torrance, CA, USA) was eluted with a mobile phase at a flow rate of 1.0 ml/min. The mobile phase consisted of 45% acetonitrile in 1% of acetic acid. The UV detector was set at 260 nm. The retention time of biochanin A and the internal standard was 11 min and 13 min, respectively. The calibration curve of biochanin A was linear within the concentration range of 10–1000 ng/ml and the limit of detection was 10 ng/ml.

2.2.5. Animal studies

Male Sprague-Dawley rats (7–8 weeks old, 230–310 g) were purchased from Samtako Bio Co., Ltd (Osan, Korea) and given free access to the normal standard chow diet (Superfeed Co, Wonju, Korea) and tap water. All animal studies were carried out in accordance with the 'Guiding Principles in the Use of Animals in Toxicology' adopted by the Society of Toxicology (USA). Rats were fasted for 24 h prior to the experiments and given free access to tap water. The rats were divided into three groups (n = 4 per group). Biochanin A in the different formulations was orally given to each group of rats at the dose equivalent to 20 mg/kg of biochanin A: Group 1 (given with pure biochanin A in 0.5% aqueous methylcellulose), Group 2 (given with 1:5:5 SD) and Group 3 (given with 1:10:10 SD). Blood samples were collected from the femoral artery at 0.25, 0.5, 0.75, 1, 2, 4, 8, 12 and 24 h following an oral administration of each formulation. Blood samples were centrifuged at

Table 1
Solubility of biochanin A from various SD formulations (mean ± S.D., $n = 3$).

Formulations (w/w)	Solubility (μg/ml)
Biochanin A (untreated powder)	6.73 ± 0.36
Biochanin A:Solutol® HS15 = 1: 5	137.27 ± 8.12
Biochanin A:Povidone K-30 = 1:5	76.61 ± 5.36
Biochanin A:Poloxamer 407 = 1:5	36.55 ± 5.49
Biochanin A:HPMC 2910 = 1:5	35.26 ± 2.64
Biochanin A:polyethylene glycol 3400 = 1:5	23.46 ± 2.83
Biochanin A:mannitol = 1:5	7.73 ± 0.62

Table 2
Solubility of biochanin A from SD formulations with Solutol® HS15 and HPMC 2910 at the various drug-carrier ratios (mean ± S.D., $n = 3$).

Formulations (w/w)	Solubility (μg/ml)
Biochanin A (untreated powder)	6.73 ± 0.36
1:1:1 SD	56.65 ± 9.27
1:3:3 SD	106.80 ± 1.83
1:5:5 SD	149.49 ± 9.31
1:10:10 SD	244.53 ± 9.60
1:20:20 SD	402.67 ± 19.47

13,000 rpm for 5 min and the obtained plasma samples were stored at −80 °C until analyzed by HPLC assay.

2.2.6. Pharmacokinetic analysis

Noncompartmental analysis was performed by using Kinetica version 5.0 (Thermo Fisher Scientific Inc., Waltham, MA). The area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal method. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were observed values from the experimental data.

2.2.7. Statistical analysis

All the mean values were presented with their standard deviations (mean ± S.D.). The statistical analysis was conducted using a one-way ANOVA followed by Dunnett's test and $p < 0.05$ was considered statically significant.

3. Results and discussion

3.1. Carrier screening and solubility tests

SDs of biochanin A was prepared by using polymeric or non-polymeric carriers at the drug–carrier ratio of 1:5 and then their effectiveness in improving the solubility of biochanin A was evaluated (Table 1). Due to the high melting point of biochanin A (about 215 °C), SD was prepared by using the solvent method rather than the melting method. As summarized in Table 1, among the tested carriers, Solutol® HS15 was most effective to increase the solubility of biochanin A by approximately 20 folds. Incorporation of drugs into micelles by using a solubilizing agent is often a favorable and simple method to dissolve the poorly water soluble drugs. However, the solubilizing agents should meet the certain criteria such as high physiological tolerance and effectiveness in drug solubilization. Solutol® HS15 exhibited favorable safety profiles with relatively low toxicity as suggested by the oral LD_{50} of approximately 20 g/kg in rats (Wade and Weller, 1994). Furthermore, Solutol® HS15 forms the loose and porous spherical micelles with a low package density (Ruchatz and Schuch, 1998). Considering that drugs can be located in the core or within pores and gaps of the loose micelles, the micelle dimensions remained unchanged and the loaded drugs are unlikely to influence the physicochemical properties of the micelles (Ruchatz and Schuch, 1998). This might be the reason for the high solubilizing capacity of Solutol® HS15 for a wide range of drugs. Therefore, Solutol® HS15 could be an effective solubilizer for biochanin A as also supported by the data in Table 1. As a result, in the present study, Solutol® HS15 was selected as a hydrophilic carrier possessing surface active properties. Furthermore, in order to overcome the drawbacks of semi-solid formation in case only Solutol® HS15 was used for the SD preparation, HPMC is selected as an additional hydrophilic polymer. HPMC is a water-soluble non-ionic cellulose ether and stable over the pH range 3.0–11.0 (Li et al., 2005). The use of Solutol® HS15/HPMC 2910 blend can also combine the profits of HPMC (recrystallization inhibition, stability, processability) (Bley et al., 2010; Hasegawa et al., 2005; Usui et al., 1997) and thus, the mixture of Solutol® HS15 and HPMC 2910 was examined for the SD formulation of biochanin A in the subsequent studies.

As summarized in Table 2 and Fig. 1, the solubility of biochanin A was significantly ($p < 0.05$) enhanced in both PMs and SDs as the proportions of Solutol® HS15 and HPMC 2910 were increased. Given that all the samples used in this study had the concentration of Solutol® HS15 higher than its critical micelles concentration (CMC) (0.021%, w/v) (Buszello et al., 2000), there should be simultaneous increase in micelle concentration along with the increase in concentration of Solutol® HS15. Therefore, solubility of biochanin A was enhanced as the concentration of Solutol® HS15 in PMs and SDs increased. However, SDs produced significantly ($p < 0.05$) higher enhancement in the solubility of biochanin A compared to corresponding PMs, implying that in addition to the solubilizing effect of hydrophilic carriers, the change in drug crystallinity to amorphous state might also have a contribution to the solubility enhancement in SDs. Therefore, the drug crystallinity was examined as described below.

3.2. X-ray diffraction (XRD)

The crystallinity of biochanin A in PM and SDs was examined by XRD patterns. The X-ray diffractograms obtained from pure biochanin A, Solutol® HS15, 1:3:3 PM and SDs at various drug–carrier ratios were illustrated in Fig. 2. Biochanin A had several dominant peaks at 2θ angles within 30°. Except for the peak at 23.3° overlapped with the peak of Solutol® HS15, biochanin A showed characteristic peaks at 11.2°, 12.1°, 16.4°, 17°, and 26.3°. Those characteristic peaks of biochanin A were still observed from the 1:3:3 PM, implying that biochanin A existed as a crystalline form in this PM. On the other hand, in the case of SDs, the characteristic peaks of biochanin A were disappeared completely, suggesting the change of drug crystallinity to amorphous form in SDs. This result also supported the greater enhancement of drug solubility from SDs compared to those from PMs in Fig. 1.

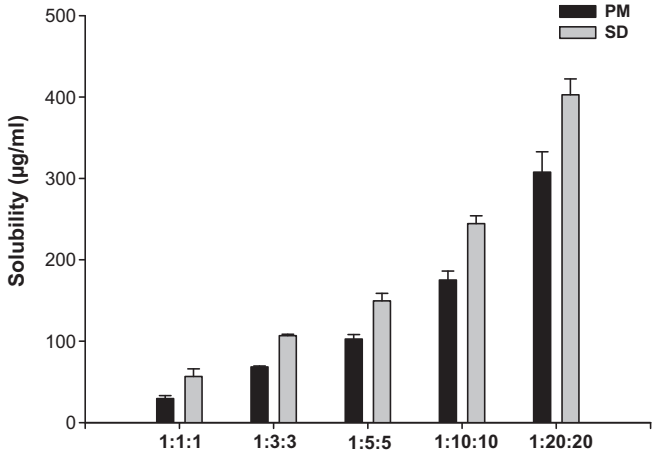

Fig. 1. Solubility of biochanin A in PMs and SDs with Solutol® HS15 and HPMC 2910 at the various drug-carrier ratios (mean ± S.D., $n = 3$).

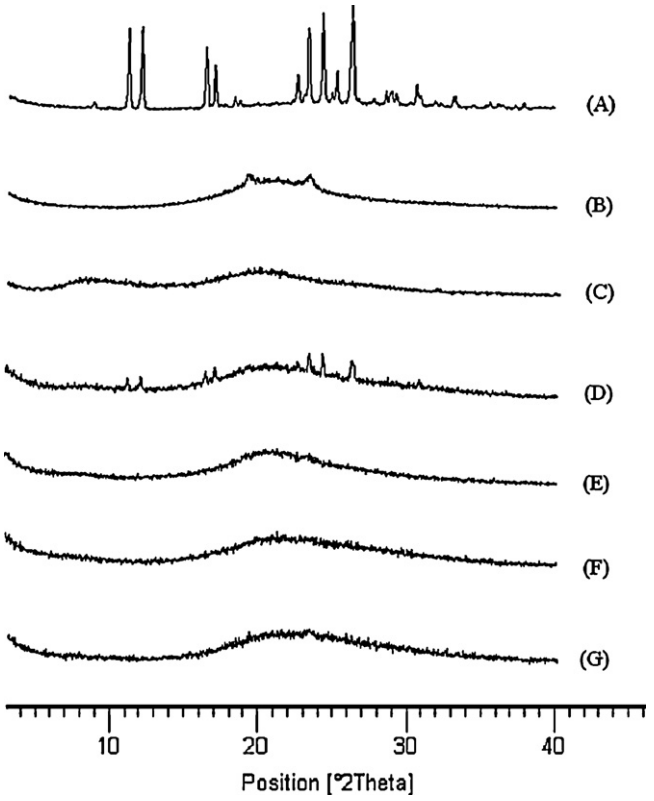

Fig. 2. X-ray diffraction patterns of (A) biochanin A, (B) Solutol® HS15, (C) HPMC 2910, (D) 1:3:3 PM, (E) 1:3:3 SD, (F) 1:5:5 SD and (G) 1:10:10 SD.

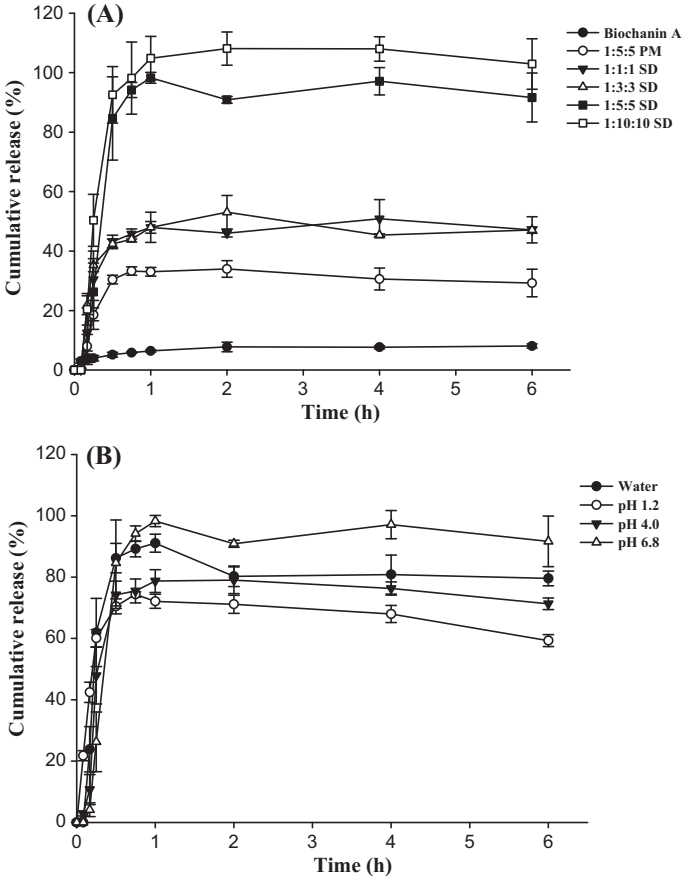

Fig. 3. (A) Dissolution profiles of biochanin A from different formulations at pH 6.8 and (B) dissolution profiles of 1:5:5 SDs at different pHs (mean ± S.D., $n = 3$).

3.3. Dissolution tests

The dissolution profiles of biochanin A, PM and SDs of various drug–carrier ratios were evaluated in pH 6.8 buffer. As shown in Fig. 3(A), biochanin A was hardly dissolved in the dissolution medium for 6 h (<10%). The dissolution of biochanin A from 1:5:5 PM was higher than that of pure biochanin A, which might be due to the drug solubilizing effect of hydrophilic carriers, however, it was still about 30%. In the case of SDs, the dissolution rates and the extent of drug release were significantly ($p < 0.05$) improved compared to the pure biochanin A or PM. Particularly, SDs (drug:Solutol® HS15:HPMC 2910 = 1:5:5 or 1:10:10) achieved the rapid and almost complete drug release (approximately 100% within 1 h) at pH 6.8. Furthermore, the pH-dependency in dissolution profiles was also examined with 1:5:5 SDs as summarized in Fig. 3(B). As pH of the dissolution medium decreased, the dissolution of biochanin A slightly decreased. Hence, the additional solubility test was performed to investigate the pH-dependency in the solubility of biochanin A and found that the solubility of biochanin A could be decreased according to the pH decrease of medium (data not shown). Therefore, the slight decrease in the dissolution of SDs at lower pH might be caused by the recrystallization and partial precipitation of biochanin A in acidic conditions.

3.4. Stability tests

SDs might be an effective formulation to improve the solubility of poorly soluble drugs but it may have a critical drawback of stability such as recrystallization. Hence, the stability of SDs was examined by periodical evaluation of the aqueous solubility and XRD patterns of 1:5:5 and 1:10:10 SDs under 4 °C and 25 °C storage conditions. As shown in Fig. 4, the solubility of biochanin A from 1:5:5 SD to 1:10:10 SD appeared to be about 150 μg/ml and 245 μg/ml at the beginning and was maintained during 3 month-storage at both 4 °C and 25 °C. Furthermore, the XRD patterns of 1:5:5 and 1:10:10 SDs after 3 months were similar to those of SDs at day 0 (Fig. 5). Those results indicated that the amorphous state of biochanin A in SDs could remain almost unchanged for 3 months. Considering that HPMC 2910 has recrystallization inhibition effect (Hasegawa et al., 2005; Usui et al., 1997) and Solutol® HS15 has an excellent drug solubillization capacity, the combined use of Solutol® HS15 and HPMC 2910 may be helpful to maintain the stability of SDs.

3.5. Pharmacokinetic studies

As 1:5:5 and 1:10:10 SDs significantly improved the solubility and the dissolution rate of biochanin A, the effect of SD formulation on the oral exposure of biochanin A was examined in rats. Mean plasma concentration–time profiles of biochanin A were evaluated in rats after an oral administration (20 mg/kg) of pure biochanin A or SDs and summarized in Fig. 6. The pharmacokinetic parameters were also determined and summarized in Table 3. As illustrated in Fig. 6, the plasma concentration–time profiles of biochanin A

Table 3
Pharmacokinetic parameters of biochanin A following an oral administration of biochanin A (20 mg/kg) in different formulations to rats (mean ± S.D., $n = 4$).

Parameter	Biochanin A	1:5:5 SD	1:10:10 SD
T_{max} (h)	1.88 ± 1.55	0.35 ± 0.22	0.25
C_{max} (ng/ml)	50.4 ± 4.27	286 ± 129*	662 ± 108*
AUC (ng h/ml)	819 ± 128	1390 ± 302	3880 ± 2490*

* $p < 0.05$, compared to the control group (pure biochanin A).

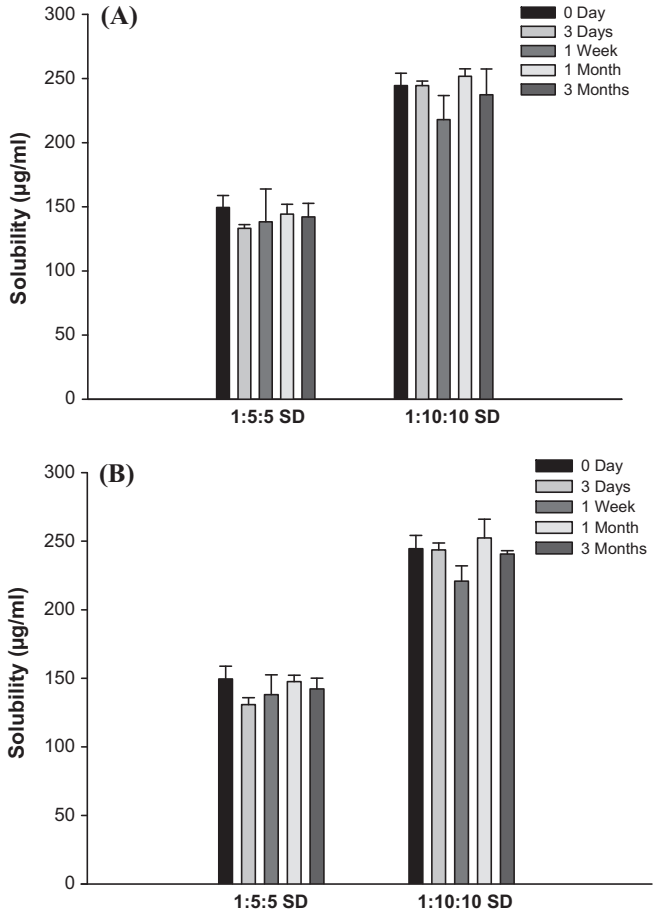

Fig. 4. Stability of SD formulations at 4 °C (A) and 25 °C (B) (mean ± S.D., $n = 3$).

indicated double peak phenomenon, which might be explained by the enterohepatic recirculation of biochanin A following the formation of glucuronide conjugates (Chen et al., 2003; Jia et al., 2004; Mallis et al., 2003). While the time of peak plasma concentration (T_{max}) of 1:5:5 SD and 1:10:10 SD was not significantly

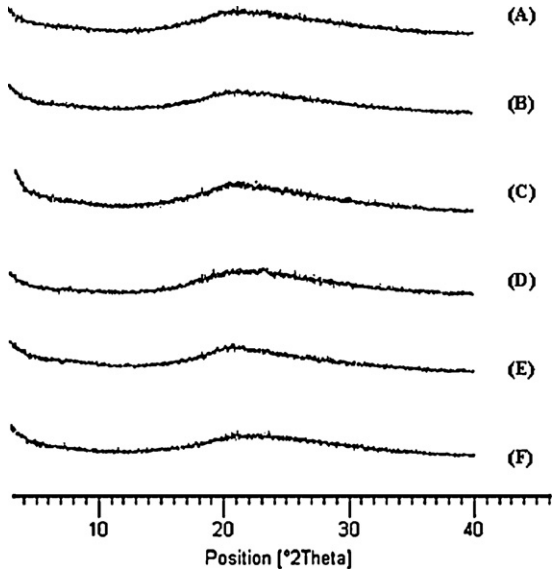

Fig. 5. X-ray diffraction patterns of (A) 1:5:5 SD at day 0, (B) 1:5:5 SD at 4 °C after 3 months, (C) 1:5:5 SD at 25 °C after 3 months, (D) 1:10:10 SD at day 0, (E) 1:10:10 SD at 4 °C after 3 months, (F) 1:10:10 SD at 25 °C after 3 months.

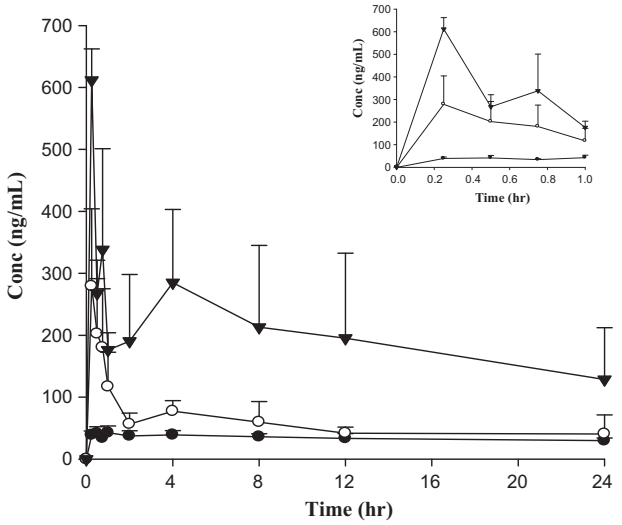

Fig. 6. Mean plasma concentration–time profiles of biochanin A after an oral administration of biochanin A (20 mg/kg) in different formulations to rats (mean ± S.D., $n = 4$). (●) Biochanin A, (○) SD (biochanin A:Solutol® HS15:HPMC 2910 = 1:5:5), (▼) SD (biochanin A:Solutol® HS15:HPMC 2910 = 1:10:10).

different from that of pure biochanin A, the peak plasma concentration (C_{max}) of 1:5:5 SD and 1:10:10 SD was significantly higher than that of pure biochanin A (approximately 5.7 and 13 folds higher for 1:5:5 SD and 1:10:10 SD, respectively). In addition, the area under the plasma concentration–time curve (AUC) of biochanin A tends to be increased via the SD formulation. Particularly, the AUC of 1:10:10 SD was enhanced significantly ($p < 0.05$, approximately 5 folds) compared to the pure biochanin A. Those results indicated that the enhanced solubility and dissolution of biochanin A via the SD formulations could lead to the improved oral bioavailability of biochanin A.

Given that the disconnect between in vitro and in vivo results of biochanin A in P-gp inhibition could be due to the low bioavailability of biochanin A (Zhang et al., 2010), the enhanced oral exposure of biochanin A via the SD preparation may lead to the improved in vivo performance of biochanin A as a P-gp inhibitor. Furthermore, as Solutol® HS15 itself has an inhibition effect on P-gp activity (Coon et al., 1991), the present SD formulation containing Solutol® HS15 might have a synergistic effect on P-gp inhibition of biochanin A. Therefore, the effect of biochanin A loaded-SD formulation on the pharmacokinetics of P-gp substrates should be further characterized in future studies.

4. Conclusion

Solid dispersion with the mixture of Solutol® HS15 and HPMC 2910 appeared to be effective to enhance the solubility and dissolution of biochanin A. Furthermore, pharmacokinetic studies in rats indicated that SD formulation significantly improved the oral exposure of biochanin A. Therefore, SD preparation with combined use of Solutol® HS15 and HPMC 2910 may be a promising approach to enhance the solubility and bioavailability of biochanin A.

Acknowledgements

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