International Journal of Pharmaceutics 415 (2011) 89–94

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

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

Hyo-Kyung Han [a,*], Beom-Jin Lee [b], Hyoung-Kyu Lee [c]

[a] College of Pharmacy, Dongguk University-Seoul, Pil-dong-3-ga, Jung-gu, Seoul 100-715, Republic of Korea
[b] Bioavailability Control Laboratory, College of Pharmacy, Kangwon National University, Chuncheon 200-701, Republic of Korea
[c] BK21 Project Team, College of Pharmacy, Chosun University, Gwangju, Republic of Korea

ARTICLE INFO

Article history:
Received 25 January 2011
Received in revised form 20 April 2011
Accepted 20 May 2011
Available online 27 May 2011

Keywords:
Biochanin A
Solid dispersion
Dissolution
Bioavailability

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

* Corresponding author. Tel.: +82 2 2260 3957.
E-mail address: hkhan@dongguk.edu (H.-K. Han).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.05.055

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