Journal of Chromatography B, 878 (2010) 1875–1880

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Short communication

A sensitive liquid chromatography–electrospray tandem mass spectrometric method for lancemaside A and its metabolites in plasma and a pharmacokinetic study in mice

Eun-Ha Joh[a,b], Dong-Hyun Kim[a,b,*]

[a] Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, 1, Hoegi, Dongdaemun-Ku, Seoul 130-701, Republic of Korea
[b] Department of Pharmacy, Kyung Hee University, 1, Hoegi, Dongdaemun-Ku, Seoul 130-701, Republic of Korea

ARTICLE INFO

Article history:
Received 20 January 2010
Accepted 2 May 2010
Available online 9 May 2010

Keywords:
Codonopsis lanceolata
Lancemaside A
Echinocystic acid
Metabolism
LC–MS/MS
Pharmacokinetic study

ABSTRACT

A high-performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) method employing electrospray ionization (ESI) has been developed for simultaneous determination of lancemaside A (3-*O*-β-D-glucuronopyranosyl-3β, 16α-dihydroxyolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester) and its metabolites in mouse plasma. When lancemaside A (60 mg/kg) was orally administered to mice, echinocystic acid was detected in the blood. T_{max} and C_{max} of the echinocystic acid were 6.5 ± 1.9 h and 56.7 ± 29.1 ppb. Orally administered lancemaside A was metabolized to lancemaside X (3β, 16α-dihydroxyolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester) by intestinal microflora in mice, which was metabolized to echinocystic acid by intestinal microflora and/or intestinal tissues. Human intestinal microflora also metabolized lancemaside A to echinocystic acid via lancemaside X. These results suggest that the metabolism by intestinal microflora may play an important role in pharmacological effects of orally administered lancemaside A.

1. Introduction

Lancemaside A, 3-*O*-β-D-glucuronopyranosyl-3β, 16α-dihydroxyolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl (1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester, is a triterpenoid saponin isolated from BuOH extract of the rhizome of *Codonopsis lanceolata* (family Campanulaceae) [1]. Triterpenoid saponins of the rhizome of *C. lanceolata*, which contain lancemaside A as a major compound, are identified by centrifugal partition chromatography and liquid chromatography–mass spectrometry [2–4]. The rhizome of *C. lanceolata* is been used in herbal medicines for inflammatory diseases such as bronchitis and cough in Asian countries [5–7]. Their saponins exhibit anti-inflammatory and anti-tumor effects [7,8]. We also reported that lancemaside A isolated from its BuOH fraction potently inhibited colitis via TLR-linked NF-κB activation in mice [9]. Similar to other saponins, such as ginsenoside Rb1, the absorption of orally administered lancemaside A from intestine into the blood may be difficult due to its hydrophilicity [10–13]. Thus, orally administered lancemaside A comes into contact with intestinal microflora in intestine and is metabolized to hydrophobic compounds and its metabolites may be absorbed into the blood.

Therefore, to understand its bioactive form, we performed a pharmacokinetic study of lancemaside A in mice.

2. Experimental

2.1. Chemicals, materials and reagents

Lancemaside A was isolated from *C. lanceolata* (CL) as previously reported by Joh et al. [9]. Compound K was isolated using the previously published method of Bae et al. [14]. β-D-Glucuronidase was purchased from Sigma (St Louis, MO, USA). Acetonitrile, methanol and formic acid (HPLC grade) were purchased from Samchun Chemicals (Pyeongtaek, Gyeonggi, Korea). All other reagents were of analytical grade.

2.2. Animals

Male ICR mice (24–28 g) were supplied from Orient animal breeding center (Seoul, Korea). All animals were housed in wire

* Corresponding author at: Department of Pharmacy, Kyung Hee University, 1, Hoegi, Dongdaemun-Ku, Seoul 130-701, Republic of Korea.
Tel.: +82 2 961 0359; fax: +82 2 957 5030.
E-mail address: dhkim@khu.ac.kr (D.-H. Kim).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.05.003

cages at 20–22 °C and 50 ± 10% humidity, fed standard laboratory chow (Samyang, Seoul, South Korea), and allowed water ad libitum. All experiments were performed in accordance with the NIH and Kyung Hee University guides for Laboratory Animals Care and Use and approved by the Committee for the Care and Use of Laboratory Animals in the College of Pharmacy, Kyung Hee University.

2.3. Preparation of lancemaside X, echinocystic acid and echinocystic acid 3-O-β-D-glucuronopyranoside (EAG)

For the preparation of lancemaside X (3β, 16α-dihydroxyolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl (1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester), the reaction mixture containing 2 mL of lancemaside A (10 mg/mL) dissolved in distilled water and 5 mL of β-D-glucuronidase (0.5 mg/mL) dissolved in distilled water was incubated at 37 °C for 5 h. The reaction mixture (7 mL) was extracted with BuOH. The BuOH layer was evaporated *in vacuo*, dissolved in acetonitrile, and applied to MPLC to afford lancemaside X (4 mg).

For the preparation of echinocystic acid, lancemaside A (50 mg) in 5 mL H_2O was added to 6N HCl (5 mL) and refluxed 2 h. The mixture was extracted with $CHCl_3$. The chloroform layer was evaporated *in vacuo*, dissolved in acetonitrile, and applied to MPLC to afford echinocystic acid (16 mg).

For the preparation of EAG, lancemaside A (50 mg) in 5 mL H_2O was added to 5N NaOH (5 mL) and heated in a water bath at 70 °C. The mixture was extracted with BuOH. The BuOH layer was evaporated *in vacuo*, dissolved in acetonitrile, and applied to MPLC to afford EAG (18 mg). Chromatographic separation was carried out on a Ultra-Pak C18 column (300 mm × 37 mm, 50 μm, Yamazen Co. Ltd., Japan). The linear-gradient of elution used is as follows: 0–4 h, 40–95% mobile phase A. The mobile phase A is acetonitrile whereas the mobile phase B is water, and pumped at flow rate of 4 mL/min.

2.4. Preparation of standard solutions

Stock solutions were prepared by dissolving lancemaside A, lancemaside X, EAG and echinocystic acid in acetonitrile and MeOH solvent (2:1, v/v) at a concentration of 1 ppm, and were further diluted with the same solvent to concentrations of 100 and 15 ppb. All working solutions were stored in a refrigerator (−20 °C).

2.5. HPLC–MS/MS instrumentation

LC–MS/MS analyses were performed on the Agilent G6410 Triple Quadrupole Mass Spectrometer with an electrospray ionization (ESI) source, coupled with an HPLC Agilent 1200 series (Agilent, CA, USA). Chromatographic separations of the sample were performed on a ZORBAX Eclipse XDB-C18 column (50 mm × 2.1 mm, i.d., 1.8 μm, Agilent). For elution, a linear gradient was applied from CH_3CN–H_2O (40:60, v/v) to CH_3CN–H_2O (95:5, v/v) for 10 min. The solvent flow rate was 0.3 mL/min. Mass spectra were acquired in ESI mode using nitrogen gas at a temperature of 350 °C, a flow rate 10 L/min, a nebulizer pressure of 45 psi, quadrupole temperature of 30 °C, and a capillary voltage of 4000 V. Samples (3 μL) were injected into the column using an autosampler. Quantitation was performed using the MRM of the transitions of m/z 1189.6 → 647.1 for lancemaside A, m/z 1014.6 → 471.2 for lancemaside X, m/z 647.3 → 407.3 for EAG and m/z 471.4 → 407.3 for echinocystic acid in negative mode.

2.6. Preparation of samples

Seven hundred microliters of mouse blood were centrifuged for 10 min at 4000 × *g*. To deprotonize the plasma, 300 μL acetonitrile and MeOH solution (2:1, v/v) were added to 300 μL supernatant, vortexed for 2 min and centrifuged for 10 min at 13,000 × *g*. For analysis, the LC–MS/MS system (Agilent, CA, USA) was used.

2.7. Method validation

Intra-day assays were performed using three replicates during 1 day, and inter-day assays were performed on 3 separate days. The precision was calculated as the RSD and the accuracy was expressed as the relative error [RE (calculated concentration − nominal concentration)/(nominal concentration) × 100%]. Recoveries and matrix effects at four QC levels were determined by comparing the peak areas of the pure solution with the peak areas of pre-extraction and post-extraction plasma blanks spiked with an equivalent concentration using three replicates. The short-term, storage term and freeze/thaw stability in mouse plasma was studied at 100 ppb using three replicates. All stability studies were evaluated by comparison of plasma samples stored under different conditions with freshly prepared samples.

2.8. Pharmacokinetic study

The mice were divided into 11 groups of four mice per group. Each mouse was orally administered lancemaside A at a dose of 60 mg/kg. The mice were exsanguinated from the heart at 0, 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 h after lancemaside A administration, group by group.

2.9. Analysis of digestive tract contents

The mice were divided into 5 groups of four mice per group. Each mouse was orally administered lancemaside A at a dose of 60 mg/kg. The mice were anesthetized with ether and killed at 0, 1, 3, 5, and 8 h after lancemaside A administration. The stomach, small intestine, cecum and colon were quickly removed, opened and the contents collected, group by group. The contents of each mouse were suspended in 10-fold volume of saline in a cooled tube, and centrifuged at 250 × *g* for 5 min. The supernatants were extracted with BuOH, evaporated to dryness and resuspended in 1 mL of MeOH. ESI-MS analyses were performed on a LCQ DECA XP MS (Thermo Finnigan, CA, USA) equipped with an electrospray ion source. All ion trap analyzer parameters were optimized according to the manufacturer's instructions. In ESI-MS experiments, the spray voltage was 4.5 kV in positive mode and −4 kV in negative mode under N_2 sheath gas flow at 50 arbitrary units. The capillary temperature was maintained at 275 °C. Two microliters of samples were injected into the column. Total ion chromatograms from m/z 150 to 2000 in ESI positive and negative modes were obtained. For tandem mass spectrometry, the maximum ion injection time, activation time, and isolated ion width were set to 500, 30 and 2.0 ms, respectively. The collision energy with helium was set to 30% of the radio frequency (5 V) applied to the ion trap analyzer.

2.10. Metabolism of lancemaside A by human fecal suspension

A reaction mixture containing 500 μL of lancemaside A (1 mg/mL) dissolved in distilled water and 4.5 mL of human fecal bacterial suspension was anaerobically incubated at 37 °C for 24 h. The reaction mixture (0.5 mL) was extracted with BuOH at 0, 1, 3, 5, and 8 h after incubation, evaporated to dryness and resuspended in 0.5 mL of MeOH for analysis with LC–MS/MS. LC/MS/MS analyses were performed on Agilent G6410 Triple Quadrupole Mass Spectrometer with an electrospray ionization (ESI) source, coupled with an HPLC Agilent 1200 series. Chromatographic separation of the sample was performed on a ZORBAX Eclipse XDB-C18 column