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The toxicokinetic profile of curdione in pregnant SD rats and its transference in a placental barrier system detected by LC–MS/MS

Xiang Meng [a,b], Ting Zhang [b], Ying Li [b], Qi Pan [b], Juan Jiang [b], Yongwei Luo [b], Liming Chong [b], Yang Yang [b], Sichong Xu [b], Li Zhou [b], Zuyue Sun [a,b,*]

[a] School of Pharmacy, Fudan University, Shanghai, China
[b] Department of Pharmacology and Toxicology, Shanghai Institute of Planned Parenthood Research, Shanghai 200032, China

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ABSTRACT

The objective of this study was to determine the toxicokinetic profile of curdione in pregnant SD rats as well as the transference of curdione into the fetus through the placental barrier system using LC–MS/MS. Thirteen pregnant SD rats were treated with 7, 21 and 63 mg/kg curdione once daily from gestational day 6 (GD_6) to GD_{15}. Blood samples were collected at different time points on GD_6 and GD_{15}. Maternal plasma, placental plasma, placenta tissue, amniotic fluid and fetal tissue were collected for concentration analysis after all the animals were sacrificed following one repeated dose on GD_{19}. The results indicated that C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ increased in a dose-dependent manner both on GD_6 and GD_{15}. At 7 mg/kg group, the total serum clearance value on GD_{15} was reduced to approximately 16.4% of that on GD_6, and the volume of distribution was also significantly decreased ($p < 0.05$). Curdione could be detected in the maternal plasma, placental plasma, placenta tissue, amniotic fluid and fetal tissue, and its concentration in the fetal tissue reached saturation at 21 mg/kg. In conclusion, curdione presents with the risk of accumulation in pregnant SD rats and may affect the fetus via transference through the placental barrier system.

1. Introduction

Baofukang vaginal suppository has been developed and used clinically in China to treat vaginitis more than ten years, particularly for the vaginitis treatment in pregnant women because of its high potency and low toxicity. However, it was seldomly known the toxic profile of Baofukang vaginal suppository in pregnant SD rats and its transference in a placental barrier system, which could provide valuable information for clinical use to determine whether it induces drug accumulation in the parent and/or offspring.

Curdione is a primary component of zedoary oils in Rhizoma Curcumae, which has been identified as the most active ingredient in Baofukang vaginal suppository (Manzan et al., 2003; Wang et al., 2010; Ma et al., 2012). So, curdione as a representative bioactive marker has been selected by the Chinese Pharmacopoeia, 2010 edition, for therapeutic applications for quality control and has been widely used in the treatment of fungal vaginitis, senile vaginitis, cervical erosion and many other gynecological diseases (Pharmacopoeia Commission of the People's Republic of China, 2010). The plasma concentration of curdione could be detected to predict the absorption of the Baofukang vaginal suppository. In this study, we used curdione as the representative bioactive marker for Baofukang vaginal suppository and determined the toxicokinetic profile of curdione in pregnant SD rats and the transference of curdione into the fetus through the placental barrier system using liquid chromatography mass spectrometry (LC–MS/MS).

After mating, 13 pregnant SD rats were eligible and treated with 7, 21 and 63 mg/kg curdione once daily by vaginal suppository administration from gestational day 6 (GD_6) to GD_{15}, these toxic dosages were 4.8-, 14.3- and 43.2-fold greater than that of the normal effective dosage used in female SD rats. On GD_{19}, all the animals were sacrificed following one repeated dose, and the maternal plasma, placental plasma, placenta tissue, amniotic fluid and fetal tissue were collected for its concentration analysis. The results can be used to predict its potential possibility for the drug accumulation and transference into the fetus through the placental barrier system determined by LC–MS/MS.

2. Material and methods

2.1. Chemicals

Curdione ($C_{15}H_{24}O_2$, (3S,6E,10S)-6,10-dimethyl-3-propan-2-ylcyclodec-6-ene-1,4-dione, purity 99%, Fig. 1) was provided by

* Corresponding author at: School of Pharmacy, Fudan University, Shanghai, China. Fax: +86 21 6443 8949.
E-mail address: sunzy64@163.com (Z. Sun).

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the Shanghai Institution of Planned Parenthood Research (Shanghai, China) (Hikino et al., 1967; Yan et al., 2005; Deng et al., 2006; Jiang et al., 2010; Hou et al., 2011; Xia et al., 2012). Glimepiride ($C_{24}H_{34}N_4O_5S$, purity 99.9%, Fig. 1) was the internal standard (IS) for the LC–MS/MS and was provided by Dr. Reddy's Laboratories Ltd. (Hyderabad, India) (Bansal et al., 2008; Kundlik et al., 2012). The methanol and acetonitrile (ACN) were HPLC-grade and were purchased from DIKMA (Illinois, USA). The formic acid (FA) was HPLC-grade and was obtained from CNW Technologies GmbH (Dusseldorf, Germany). Ethyl acetate was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and was analytical grade. All other reagents were analytical grade. Water was purified by reverse osmosis. All solutions were filtered through a 0.22-μm nylon filter membrane manufactured by Millipore Corporation (Bedford, MA).

2.2. Instruments

The AB SCIEX Triple Quad™ 5500 tandem mass spectrometer was manufactured by AB Sciex (Massachusetts, USA). The LC system was manufactured by Shimadzu (Kyoto, Japan) and included two pumps (LC-20AD), an automated sample injector (SIL-HTc), a vacuum degasser (DGU-20A3) and a variable wavelength detector. The CAPCELL C_{18} MGIII (100 × 2.0 mm, 5 μm) was from Shiseido and was used as an RP-HPLC column (Japan). The guard column was C18 (4.0 × 3.0 mm, 5 μm) from Phenomenex (California, USA). The data analysis and processing were conducted with the Analyst 1.5.1 software from AB Sciex.

2.3. Animal experiment

Eighteen female SD rats weighing 180–200 g were purchased from BK Laboratory Animal Co., Ltd. (Shanghai, China). The rats were raised under standard laboratory conditions of room temperature (22 ± 2 °C) and relative humidity (50 ± 10%), with a 12 h light–dark cycle. Water and food were provided *ad libitum*. After 5–7 days of acclimation period, eighteen female SD rats were randomly assigned to 7, 21 and 63 mg/kg curdione group and be mated. The first mating day was recorded as gestational day 1 (GD_1). On GD_6, thirteen female SD rats had been found pregnant and eligible for the research purpose. The animal number of three doses was 4, 3, and 6, respectively. All the procedures were approved by the Ethical Committee of the Shanghai Institution of Planned Parenthood Research and every effort was made to minimize stress to the animals.

2.4. Conditions for the detection method

The LC–MS/MS conditions were as follows: the mobile phase 1 was 0.5% FA solution (A) – 0.5% FA-ACN solution (B), and the gradient elution of mobile phase 1 was conducted at a flow rate of 0.3 mL/min into the LC–MS/MS. The mobile phase 2 was 0.02% FA – 50% ACN solution, which was used for reduce impurities. A gradient elution programme of mobile phase was conducted for chromatographic separation as follows: 0–4 min (mobile phase 2), 4–6.0 min (50–50% B of mobile phase 1), 6.0–7.9 min (50–100% B of mobile phase 1), 7.9–9.5 min (mobile phase 2). The temperatures of the column and autosampler were 30 °C and 4 °C, respectively. Positive ion mode was used for both the sample and the IS. The Turboionspray™ interface and ion spray voltage were 550 °C and 5500 V, respectively. Pressures of 60, 60, 35, and 10 psi were used for the nebulizer gas, auxiliary gas, curtain gas and collision gas, respectively. For curdione, 23, 95, 11, and 6 V were used for the collision energy (CE), declustering potential (DP), collision cell exit potential (CXP) and entrance potential (EP), respectively. Additionally, for glimepiride, 95, 18, 4, and 10 V were used, respectively. Multiple reaction monitoring (MRM) mode was used to detect the parent-to-daughter ion tran-

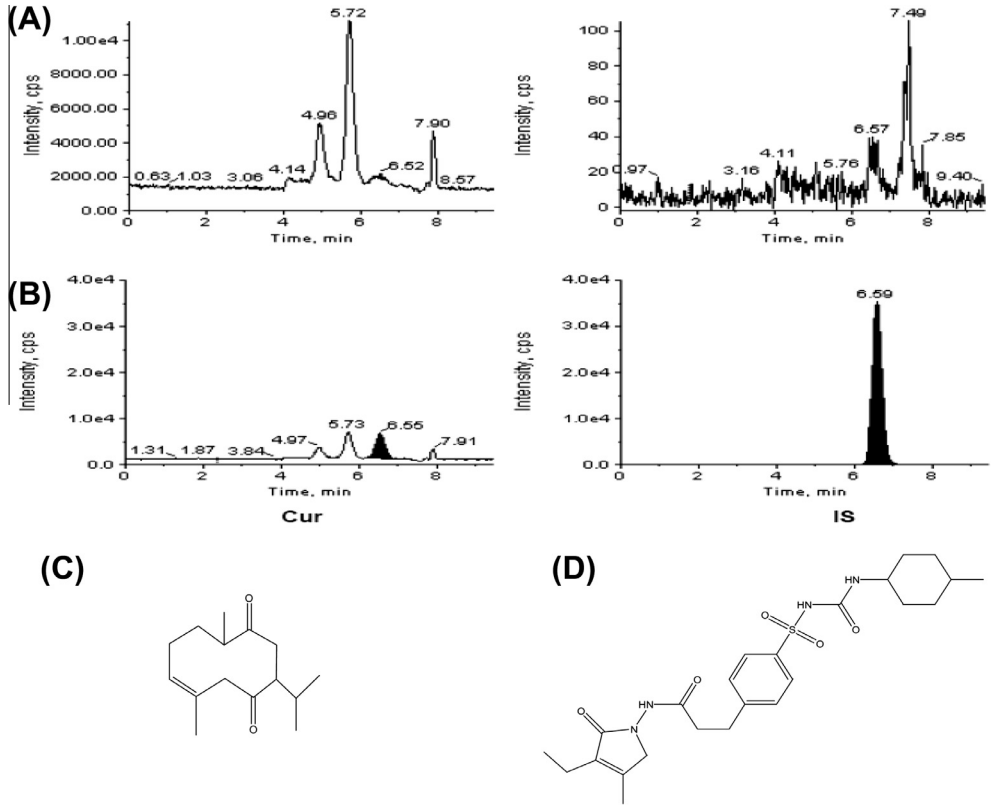

Fig. 1. Typical MRM chromatograms of pregnant SD rats. Blank plasma (A) and an LLOQ (0.5 ng/mL) sample with the IS (50 ng/mL) (B). Chemical structures of curdione (C) and glimepiride (D).