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Paeoniflorin and liquiritin, two major constituents in Chinese herbal formulas used to treat hyperprolactinemia-associated disorders, inhibits prolactin secretion in prolactinoma cells by different mechanisms

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ABSTRACT

Ethnopharmacological relevance: Paeoniflorin and liquiritin are major constituents in some Chinese herbal formulas, such as Yiru Tiaojing (YRTJ) Granule (a hospitalized preparation) and Peony-Glycyrrhiza Decoction, used for hyperprolactinemia-associated disorders.

Aim of the study: To investigate the effect of paeoniflorin and liquiritin on prolactin secretion.

Materials and methods: The effect of YRTJ Granule on metoclopramide-induced hyperprolactinemia was tested in rats. Paeoniflorin and liquiritin in the YRTJ Granule extract were identified and quantified by HPLC. The effects of paeoniflorin and liquiritin on prolactin secretion were examined in prolactinoma cells that were identified morphologically and by Western blot. The concentration of prolactin was determined by ELISA. The gene expression was analyzed by Western blot.

Results: YRTJ Granule ameliorated metoclopramide-induced hyperprolactinemia in rats. The contents of paeoniflorin and liquiritin in YRTJ Granule were 7.43 and 2.05 mg/g extract, respectively. Paeoniflorin, liquiritin and bromocriptine (a dopamine D_2 receptor (D_2R) agonist) decreased prolactin concentration in MMQ cells expressing D_2R. However, the effect of liquiritin and bromocriptine was abolished in GH3 cells lacking D_2R expression. Interestingly, paeoniflorin still decreased prolactin concentration in GH3 cells in the same manner. Furthermore, paeoniflorin suppressed prolactin protein expression, and was without effect on D_2R protein expression in both MMQ and GH3 cells.

Conclusions: The present results suggest that paeoniflorin and liquiritin play a role in YRTJ Granule-elicited improvement of hyperprolactinemia. While the effect of liquiritin is D_2R-dependent, paeoniflorin D_2R-independently inhibits prolactin secretion in prolactinoma cells that may especially benefit the hyperprolactinemic patients who are refractory to dopaminergic therapies.

1. Introduction

Although the human prolactin, a polypeptide hormone mainly produced by pituitary lactotrophs, has numerous biological activities, pathological hyperprolactinemia produces many diseases including reproductive disorders, such as amenorrhea or oligomenorrhea, galactorrhea, and infertility in women and impotence and galactorrhea in men (Freeman et al., 2000). Pathological hyperprolactinemia is a well-recognized condition that occurs due to prolactinoma, hypothalamic or pituitary tumors compressing the pituitary stalk, drugs such as antipsychotics, hypothyroidism, or hepatorenal severe disorders (Freeman et al., 2000).

Empirical evidence suggests that many herbal formulas have the therapeutic potential for hyperprolactinemia (Zhang et al., 2010). Treatment with Yiru Tiaojing (YRTJ) Granule, the herbal preparation used in Nanfang Hospital, Southern Medical University, China, for

Abbreviations: D_2R, dopamine D_2 receptors; MCP, metoclopramide; TGF, transforming growth factor; TNF, tumor necrosis factor; YRTJ, Yiru Tiaojing

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hyperprolactinemia-associated disorders for over 10 years, has been demonstrated to improve hyperprolactinemia in patients (Wang et al., 2009). Paeoniae-Glycyrrhiza Decoction (an ancient traditional Chinese herbal formula) and its related formulas have been widely used to treat various hyperprolactinemia-associated disorders, such as infertility (Yaginuma et al., 1982), ovulation disorders (Takahashi and Kitao, 1994, 1988), neuroleptic-induced hyperprolactinemia (Yamada et al., 1996, 1997) and amenorrhea (Yamada et al., 1999), gonadotropin-releasing hormone agonist therapy-induced menopausal symptoms (Tanaka, 2001), hyperprolactinemic impotence (Xu, 2003), antipsychotic-induced sexual dysfunction in schizophrenia (Costa et al., 2006), and risperidone-induced hyperprolactinemia in patients with schizophrenia (Yuan, 2008). Radix *Paeoniae alba* and Radix et Rhizoma *Glycyrrhiza* are two principal components in the formulas mentioned above. Paeoniflorin (Fig. 2A) is a major ingredient in Radix *Paeoniae alba* and Radix *Paeoniae rubra* (another species also commonly used in the gynecology of traditional Chinese medicine) (> 1.6% and 1.8% in dried raw materials, respectively) (Chinese Pharmacopoeia Commission, 2015). Liquiritin is a major constituent of Radix et Rhizoma *Glycyrrhiza* (> 0.5% in dried raw material) (Chinese Pharmacopoeia Commission, 2015). Although YRTJ Granule (Wei et al., 2015) and Paeoniae-Glycyrrhiza Decoction (Wang et al., 2012, 2015) have been demonstrated to improve hyperprolactinemia in vivo and in vitro, It is still unknown whether paeoniflorin and liquiritin play a role.

In the present study, we investigated the effect of paeoniflorin and liquiritin on prolactin secretion in two distinct prolactinoma cells: MMQ cells and GH3 cells.

2. Materials and methods

2.1. Chemicals and reagents

Paeoniflorin ($C_{23}H_{28}O_{11}$, MW: 480.45, purity > 98%) and liquiritin ($C_{21}H_{22}O_9$, MW: 418.39, purity > 98%) (Dalian Meilun Biological Technology CO., LTD, Dalian, China), and bromocriptine (2-Bromo-α-ergocryptine methanesulfonate salt, $C_{32}H_{40}BrN_5O_5{\cdot}CH_4SO_3$, MW: 750.70, purity > 98%, Sigma-Aldrich, Guangzhou, China) were purchased commercially.

2.2. Preparation and identification of YRTJ formula extract

The dry raw herbs used in the preparation of YRTJ formula were purchased from Shenzhen Hong'en Medicine Co., Ltd., Shenzhen, China, and identified by Dr Zhihua Song, a botanist in Nanfang Hospital, Southern Medical University, Guangzhou, China. The voucher specimens were deposited in the pharmacy of Nanfang Hospital. YRTJ formula extract was supplied by the hospital (Batch number: 20151211). The YRTJ formula contains a mixture of four herbs: *Rhizoma Curculiginis* (*Curculigo orchioides* Gaertn., golden eye-grass, xiānmáo in pinyin, voucher specimen No: NFY00057; 22.7%), *Radix Morindae officinalis* (*Morinda officinalis* How, morinda root, bājǐ in pinyin, voucher specimen No: NFY00058; 22.7%), *Radix Paeoniae alba* (*Paeonia lactiflora* Pall., root of herbaceous peony, báisháo in pinyin, voucher specimen No: NFY00059; 27.3%) and *Radix et Rhizoma Glycyrrhizae* (*Glycyrrhiza uralensis* Fisch., licorice root, gāncǎo in pinyin, voucher specimen No: NFY00060; 27.3%). To prepare the extract, the herbs were ground into crude powder and extracted with purified water three times (10 volumes of water for 2 h boiling, 8 volumes of water for 1.5 h boiling, and 6 volumes of water for 1 h boiling, respectively). The combined filtrate was evaporated under reduced pressure below 60 °C. The yield of the extract was 20%. For quality control, paeoniflorin and liquiritin in YRTJ formula extract were identified and quantified by the HPLC method recommended by Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2015). Briefly, a HPLC profile was performed on a Shimadzu SPD-20A variable wavelength instrument with Dual UV Detector and SIL-20A autoinjector operated through a Shimadzu CBM-20A communication module and with LC solution workstation software. The chromatography was carried out on a Phenomenex Luna 5 μm, 250 mm, 4.6 mm, C18 column (30 ℃) at a flow rate of 1.0 mL/min with detection at 231 nm. The sample injection volume was 20 μl (0.5 g test sample extracted in 50 mL methanol with ultrasonic). The mobile phase gradient consisted of a mixture of acetonitrile and 0.1% (v/v) phosphoric acid water solution (0–17 min, 18:82; 19–29 min, 45:55; 31–45 min, 18:82). The major peaks in YRTJ extract were identified as paeoniflorin and liquiritin by comparison of the retention times. The contents of paeoniflorin and liquiritin were quantified by comparison of the area under curve of the sample with an injection of a mixture standard solution of themselves.

2.3. Animal and treatment protocols

All animal procedures were conducted according to international, national and institutional rules regarding animal experimentation, and approved by the Animal Ethics Committee, Southern Medical University, China.

Animal experimentation was conducted as described previously (Wei et al., 2015). Briefly, female Sprague–Dawley rats weighing 180–220 g and the standard chow were supplied by the Animal Experiment Centre of Southern Medical University Ltd., China. Rats were housed in a temperature controlled facility (21 ± 1 °C, 55 ± 5% relative humidity) with a 12-h light/dark cycle. Animals were allowed free access to water and the standard chow for at least 1 week prior to starting the experiments.

In clinic, YRTJ granule is given orally at 20 g daily for one month as a therapeutic period, which may continue for three periods. According to the exchange rate from humans to rats, 2.1 g/kg and 4.2 g/kg, which were equal to one and two times of human dosage, respectively, were used in the present study. To establish the model of hyperprolactinemia, 40 animals were injected (i.p.) with metoclopramide (MCP, 75 mg/kg, twice daily) for 15 days. Additional 8 rats were injected with saline as the control group. On the day 16, fasted (overnight) blood samples were collected under chloral hydrate anesthesia for determination of serum prolactin concentration by ELISA (Uscm, Life Science, Wuhan, China). 32 MCP-treated rats with hyperprolactinemia (> 25 ng/mL) were selected and divided according to the concentration of serum prolactin into 4 groups as follows (8 each): MCP control, MCP YRTJ 2.1 g/kg and MCP YRTJ 4.2 g/kg, and MCP bromocriptine. Animals in MCP YRTJ- and MCP bromocriptine-treated groups were administered YRTJ 2.1 or 4.2 g/kg, or bromocriptine 0.5 mg/kg (suspended in 5% Gum Arabic solution, by gavage once daily), respectively. The rats in the corresponding normal- and MCP-control groups received vehicle (5% Gum Arabic) alone. All rats had free access to the standard chow. At the end of day 45, fasted serum prolactin concentration was determined again to evaluate the effects of the treatments. In addition, serum luteinizing hormone and estradiol concentrations were also determined by ELISA (Uscm, Life Science, Wuhan, China).

2.4. Cell culture

MMQ cell line was purchased from the Cell Culture Centre, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, China, and cultured in F12 (GIBCO) containing 2.5% fetal bovine serum and 15% horse serum. GH3 cell line was purchased from the Cell Culture Centre of Sun Yat-sen University, China, and cultured in F12K (GIBCO) containing 2.5% fetal bovine serum and 15% horse serum. The cell lines passage 2–3 times in a week.

2.5. Morphological identification of cells

Log-phase MMQ cells and GH3 cells were photographed using a light microscope at 100× magnification and identified morphologically.