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Research paper

Studies on the regulatory effect of Peony-Glycyrrhiza Decoction on prolactin hyperactivity and underlying mechanism in hyperprolactinemia rat model

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HIGHLIGHTS

- The hyperprolactinemia rat model is established successfully.
- The anti-hyperprolactinemia activity of Peony-Glycyrrhiza Decoction is confirmed.
- PGD modulates the expression of DRD_2 and DAT to suppress the prolactin level.
- PGD restores serum progesterone level to suppress the hyper-level of prolactin.
- Peony play a more important role in PGD-mediated anti-hyperprolactinemia effect.

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ABSTRACT

Clinical trials have demonstrated the beneficial effects of Peony-Glycyrrhiza Decoction (PGD) in alleviating antipsychotic-induced hyperprolactinemia (hyperPRL) in schizophrenic patients. In previous experiment, PGD suppressed prolactin (PRL) level in MMQ cells, involving modulating the expression of D_2 receptor (DRD_2) and dopamine transporter (DAT). In the present study, hyperPRL female rat model induced by dopamine blocker metoclopramide (MCP) was applied to further confirm the anti-hyperpPRL activity of PGD and underlying mechanism. In MCP-induced hyperPRL rats, the elevated serum PRL level was significantly suppressed by either PGD (2.5–10 g/kg) or bromocriptine (BMT) (0.6 mg/kg) administration for 14 days. However, in MCP-induced rats, only PGD restored the under-expressed serum progesterone (P) to control level. Both PGD and BMT administration restore the under-expression of DRD_2, DAT and TH resulted from MCP in pituitary gland and hypothalamus. Compared to untreated group, hyperPRL animals had a marked reduction on DRD_2 and DAT expression in the arcuate nucleus. PGD (10 g/kg) and BMT (0.6 mg/kg) treatment significant reversed the expression of DRD_2 and DAT. Collectively, the anti-hyperPRL activity of PGD associates with the modulation of dopaminergic neuronal system and the restoration of serum progesterone level. Our finding supports PGD as an effective agent against hyperPRL.

1. Introduction

Hyperprolactinemia (hyperPRL), a clinical hypothalamic-hypophysis disorder, defined as an abnormally high level of prolactin (PRL) in blood (≥25 ng/ml for women and ≥20 ng/ml for men), and is a notable adverse effect of antipsychotic therapy [10,12]. Antipsychotic-enhanced PRL secretion correlated with the acute and chronic dopamine (DA)-blocking actions [9]. Although DA agonists such as bromocriptine (BMT) are administrated for antipsychotic-induced high PRL [6], the side effects including aggravated psychosis and abnormal involuntary are a greater risk for patients than hyperPRL itself [6]. Identifying alternative therapy for hyperPRL is highly desired.

An herbal medicine formula Paeoniae-Glycyrrhiza Decoction (PGD) made from Paeonia (PR) and Glycyrrhiza radices (GR) has been introduced into the treatment in ovulation disorders,

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menopause and hyperPRL [18–19,23]. In our clinical trial, a great proportion of patients show improvements on risperidone-induced adverse effects associated with hyperPRL after PGD administration [23]. Further *in vitro* and *in vivo* experiments demonstrate that the hyper-level of PRL in MMQ cells and hyperprolactimia rat model is significantly reduced after PGD administration [22].

These encouraging results led us to further study the mechanisms underlying anti-hyperPRL effects of PGD in hyperprolactimia rat model. It is considered that pituitary PRL secretion is regulated mainly through the hypothalamic neuro-endocrine dopaminergic system [9]. Tyrosine hydroxylase (TH) is an enzyme that catalyzes the rate-limiting step of tyrosine conversion in the biosynthesis of DA, and is one of the targets of PRL action within the hypothalamus [2,16]. In addition, dopamine transporters (DAT), responsible for re-uptaking of DA within hypothalamus, is also a component in the overall regulation of PRL homeostasis. D_2 receptor (DRD_2) shows an important effect on short-loop feedback mechanism of PRL regulation, and the robust PRL expression is observed in DRD_2-null mice [4]. Moreover, progesterone (P) stimulates the production of deciduas PRL, conversely, it appears to be a potent inhibitor of myometrial PRL production [11].

In the present study, we examined the effects of PGD on the concentration of serum PRL and progesterone. The expression of DRD_2, DAT and TH in pituitary gland and hypothalamus after PGD administration were determined. Our results revealed that PGD displayed anti-hyperPRL effects not only through the regulation of dopamine mediators, but also through the normalization of other sexual hormone dysfunction, including progesterone.

2. Materials and methods

2.1. Herbal preparation

The raw materials of Paeoniae and Glycyrrhiza radices that constitute the PGD formula were supplied by the Pharmacy of School of Chinese Medicine in the University of Hong Kong (HKU). The extraction was conducted in an automatic boiling machine. Sliced, broiled Paeoniae and Glycyrrhiza radices (50 g each) were immersed and boiled in a 10-fold volume of distilled water for 2 h. This process was repeated twice as previously reported [22]. The extractive solution was pooled, concentrated, and freeze-dried into a ratio of 1:5 in weight of powder to raw materials for the herbal preparation.

2.2. HyperPRL rat model establishment and drug administration

Experimental protocol was approved by Committee on the Use of Live Animals in Teaching and Research (CULATR) of Li Kang Shing Faculty of Medicine of the University of Hong Kong. Sprague-Dawley female rats aged 11 weeks old and weighing 280–300 g were used in the experiment. Animals were housed in groups of two in clear plastic cages and maintained on a 12-h light/dark cycle (lights on 07:00–19:00 h) at 23 ± 1 °C with water and food available *ad libitum*. Vaginal smears were examined daily to ensure that collection of blood samples were conducted in animals in the same stage of the oestrous cycle.

Experimental hyperPRL rat model was produced similarly as previous studies [22]. 45 animals were given intraperitoneally (i.p.) metoclopramide (MCP; Sigma, USA) (50 mg/kg, three times per day), a dopamine inhibitor for 10 days. Another 9 rats were treated with 0.9% salt solution to serve as controls. At day 10, blood was drawn from the tail vein for PRL measurement. Compared to non-treated rats, all 30 MCP-treated animals displayed an at least 80% elevation of serum PRL, and all of them were used for experimental treatment and received gastric PGD at a dose of 0 g/kg, 2.5 g/kg, 5 g/kg, or 10 g/kg, or with 0.6 mg/kg BMT for 14 days in a random manner ($n = 9$/group). During the experiment, the bodyweight of each rat was monitored everyday. At the end of PGD administration, blood samples were collected and used to determine the concentration of PRL and progesterone. Tissues of pituitary gland and hypothalamus in each group were collected and used to detect the alteration of DRD_2, DAT and TH expression.

2.3. Biochemical analyses

2.3.1. Hormone assay

The concentration of progesterone in rat sera was determined *via* Elecsys 2010 (Roche, Switzerland) directly. Serum PRL concentration was measured using enzyme-linked immunosorbent assay (ELISA) (Calbiotech, USA).

2.3.2. Western blotting

Western blotting was conducted to determine the expression of DRD_2, DAT and TH in tissues of pituitary gland and hypothalamus. Tissue proteins were extracted by RIPA buffer (Sigma–Aldrich, USA) containing the 1% protease inhibitor cocktail (Sigma–Aldrich, USA) and 2% phenylmethanesulfonyl fluoride (PMSF; Sigma–Aldrich, USA). The concentration was determined *via* Bradford protein assay using coomassie brilliant blue G-250 (Bio-rad Laboratories Inc., USA). Proteins were separated by a 10% SDS-PAGE gel and transferred electrophoretically onto nitrocellulose membranes (0.45 μm, Bio Basic, Inc.). Immunodetection was performed with the primary antibodies against DRD_2, DAT, TH and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Santa cruz Biotechnology, Inc., USA) at a dilution of 1:1000 at 4 °C overnight, followed by co-incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies for 30 min at room temperature. The chemiluminescence was determined using ECL detection kits (GE Healthcare, UK). The intensity of protein bands was quantified by scanning densitometry with Quantity One 4.5.0 software.

2.3.3. Immunofluorescent examination of brain tissues

After 14-day 10 g/kg PGD and 0.6 mg/kg BMT administration, rats were anesthetized with Ketamine (100 mg/kg) and Xylazine (5 mg/kg), and then transcardially perfused with cold PBS followed by 4% paraformaldehyde for 30 min. Brains were harvested and soaked into 4% paraformaldehyde for 24 h. Further, samples were dehydrated *via* soaking in 30% sucrose for 48 h. This step was repeated for three times. After dehydration, samples were soaked into TOC in suitable containers and frozen at −80 °C. The slice of brain tissue is cut at 15 μm.

Immunofluorescence was performed to detect the expression changes of DAT and DRD_2 in arcuate nucleus of hypothalamic. The prepared sections of brain were permeabilized with 0.15% Triton X-100 (Sigma–Aldrich, USA) for 10 min, and then incubated with blocking buffer containing 5% goat serum and 0.1% Triton X-100 for 2 h at room temperature. Then the sections were incubated with primary antibodies including anti-DRD_2 and anti-DAT (1:200, Santa Cruz, USA) for 48 h at 4 °C followed with three washes with PBS. Next, sections were incubated with Alexa Fluor 568 (1:500; Invitrogen, USA) and Alexa Fluor 488 (1:1000; Invitrogen, USA) for 2 h at room temperature. Nuclei were counterstained with DAPI (100 ng/ml, Sigma Aldrich, USA) for 15 min at room temperature. Fluorescent images were obtained using a fluorescent microscope (10X; CCD camera, Axio Observer Z1; Carl Zeiss, Germany). Image J software was used to calculate the fluorescence intensity of each picture.

2.4. Statistical analysis

A One-Way variance analysis (ANOVA) was used to detect statistical significance, followed by *post-hoc* multiple comparisons