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Not only dopamine D_2 receptors involved in Peony-Glycyrrhiza Decoction, an herbal preparation against antipsychotic-associated hyperprolactinemia

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ABSTRACT

Clinical studies have demonstrated the effectiveness of an herbal preparation called Peony-Glycyrrhiza Decoction (PGD) in alleviating antipsychotic-induced hyperprolactinemia (hyperPRL). In the present study, we further examined the pharmacological action of PGD on prolactin (PRL) secretion using *in vitro* and *in vivo* models, with specific attention to the role of dopaminergic mediators and other sex hormones. Treatment with PGD at 1–5 mg/ml significantly suppressed PRL secretion and synthesis in MMQ cells, a model of hyperPRL derived from pituitary adenoma cells. The suppressive effects were completely abolished by pretreatment with 10 μM haloperidol, a dopamine D_2 receptor antagonist. Consistent with a D_2-action, PGD did not affect PRL in rat pituitary lactotropic tumor-derived GH3 cells that lack the D_2 receptor expression but significantly increased the expression of D_2 receptors and dopamine transporters (DAT) in PC12 cells. In a rat model of hyperPRL, produced by repeated injection of the dopamine blocker metoclopramide (MCP), chronic PGD (2.5–10 g/kg daily) significantly reduced elevated serum PRL. The reduction in magnitude was similar to that elicited by bromocriptine (BMT), a dopamine D_2 receptor agonist currently used for treatment of hyperPRL. Neither PGD nor BMT altered serum estradiol, but PGD reversed decreased serum progesterone to control level, whereas BMT did not. These results indicate that the anti-hyperPRL effects of PGD are associated not only with D_2 receptor and DAT modulation, but also with a normalization of other sex hormone dysfunction. This experimental evidence supports clinical use of PGD as an effective treatment of antipsychotic-induced hyperPRL.

1. Introduction

The majority of patients with chronic schizophrenia require long-term maintenance treatment (Kane and Correll, 2010). Although antipsychotic agents are the mainstay of therapy, the treatment outcomes are often unsatisfactory, and a large portion of patients experience symptom exacerbation and relapse (Kane and Correll, 2010). Adverse side effects of antipsychotics, resulting in poor medication compliance and discontinuation, substantially contribute to poor outcome (Kane and Correll, 2010). Hyperprolactinemia (hyperPRL) is a common adverse effect incurred by antipsychotic therapy, with a prevalence of estimated at 40%–70% in the schizophrenic population (Halbreich and Kahn, 2003; Inder and Castle, 2011). HyperPRL may cause amenorrhoea, galactorrhea, sexual impairments and infertility (Halbreich and Kahn, 2003). Antipsychotic-induced hyperPRL is associated with acute and chronic dopamine-blockade in the hypothalamus (Fitzgerald and Dinan, 2008). Dopamine agonists, such as bromocriptine (BMT), are considered clinically if high prolactin (PRL) levels do not improve following the reduction in antipsychotic doses (Biller et al., 1999; Marken et al., 1992). However, the addition of dopamine agonists may aggravate psychosis and precipitate abnormal involuntary movements, and may thus pose a greater risk than hyperPRL itself (Biller et al., 1999). Therefore, a search for alternative treatment is urgently needed.

Empirical evidence suggests that many herbal medicines possess the therapeutic potential to alleviate hyperPRL symptoms (Zhang et al., 2010). The herbal preparation called Peony-Glycyrrhiza Decoction (PGD), made from Paeonia and Glycyrrhiza radices, has been widely used to treat various hyperPRL-related symptoms in China and Japan (Costa et al., 2006; Xu, 2003; Yamada et al., 1996, 1997, 1999; Yuan, 2008). Case studies have reported beneficial effects of PGD and similar preparations in patients with infertility (Yaginuma et al., 1982), ovulation disorders (Takahashi and Kitao, 1994; Takahashi et al., 1988), menopause (Tanaka, 2001), and hyperPRL-related

Abbreviations: ANOVA, variance analysis; BMT, bromocriptine; CV, coefficients of variation; DAT, dopamine transporters; hyperPRL, hyperprolactinemia; MCP, metoclopramide; PGD, Peony-Glycyrrhiza Decoction; PRL, prolactin.

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impotence (Xu, 2003). Our controlled trial confirmed that PGD significantly lowered risperidone-induced elevation of blood PRL levels and produced a significantly greater improvement on hyperPRL-associated symptoms compared to standard BMT treatment (Yuan et al., 2008).

In response to these promising results from clinical studies, we planned a direct experimental test of the mechanisms underlying the anti-hyperPRL effects of PGD. Most conventional anti-hyperPRL agents reduce PRL secretion through D_2 receptor agonism in the hypothalamic neuroendocrine dopaminergic system (Fitzgerald and Dinan, 2008). However, dopamine transporters (DAT) and other sex-steroids other than PRL are also involved in the pathophysiology of hyperPRL (Demaria et al., 2000; Halbreich and Kahn, 2003). We therefore hypothesized that the therapeutic efficacy of PGD in alleviating antipsychotic-induced hyperPRL could be attributed to modulation of multiple mediators, including not only dopaminergic mediators, but also sex hormones. To test this hypothesis, we examined the effects of PGD on D_2 receptor- and DAT-mediated responses and PRL secretion in cell-culture systems; and modulation of PRL, estradiol and progesterone in the rat model of hyperPRL.

2. Materials and methods

2.1. Herbal preparation and determination of the quality

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). Water extraction was utilized for PGD preparation to preserve bioactive constituents, as recommended in the Chinese Pharmacopoeia that it is an optimal process (Chinese Pharmacopoeia Commission, 2010). The extraction was conducted in an automatic boiling machine. Sliced, broiled Paeonia 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 (Yuan et al., 2008). The extracted solution was pooled, concentrated, and freeze-dried to provide a powder in a ratio of 1:5 with raw materials. The powder was dissolved to 100 mg/ml as a stock solution for experiments.

In order to ensure the quality of the herbal preparation, 3 batches of PGD used in this study were prepared and inter-batch coefficients of variation (CV) of the contents of the 6 known constituents, albiflorin, paeoniflorin, liquiritin, liquiritigenin, glycyrrhizic acid, and glycyrrhetinic acid, were measured using reverse-phase high-performance liquid chromatography (HPLC). CVs of all constituents measured were less than 15% across the 3 batches (Fig. 1).

2.2. In vitro experiments in culture cells

2.2.1. Cell lines and culture

MMQ, GH3, and PC12 cell lines were used for *in vitro* experiments.

MMQ cells are an exemplary model of hyperPRL derived from rat pituitary adenoma cells responsive to dopamine (Judd et al., 1988). Dose-dependent and time-course responses of PRL secretion and synthesis to PGD treatment, as well as the effects of pretreatment with dopamine D_2 receptor antagonist, were evaluated in this cell line.

GH3 cells were derived from rat pituitary lactotropic tumoral cells that lack D_2 receptor expression (Giacomini et al., 2009; Missale et al., 1991). GH3 cells were used to determine if deficiency of D_2 receptors altered PGD suppression of hyperactive PRL.

Rat pheochromocytoma PC12 cells abundantly express D_2 receptors and DAT (Chiasson et al., 2006; Fazeli et al., 2011). The effects of PGD on these two dopamine mediators were further examined in PC12 cells.

All three line cells were cultured in 75 cm^2 flask in F12 medium for MMQ cells, F10 medium for GH3 cells, and Dulbecco's Modified Eagles's Medium (DMEM) for PC12 cells; supplemented with 10–12.5% heat-inactivated horse serum (HS), 5% fetal calf serum (FBS), penicillin (100 IU/ml), and streptomycin (100 μg/ml) under a humidified atmosphere containing 5% CO_2 at 37 °C. The culture medium was replaced with fresh medium every 2–3 days. Culture cells were transferred to 35 mm-diameter 6-well plates for experimental treatment when the density reached 70–80% confluence.

2.2.2. Experimental design

For dose-curve and time-course experiments, MMQ cells were treated with a range of PGD doses from 0.1 mg/ml to 6 mg/ml for 12–48 h. The culture medium was collected at different incubation time points for the measurement of PRL secretion. The cells were collected and prepared for determining cellular PRL synthesis (see below). The optimal doses and treatment duration were then determined for subsequent experiments. In a separate experiment, MMQ cells were pretreated with 10 μM haloperidol, a D_2 receptor antagonist for 30 min, followed by co-treatment with and without PGD at an effective dose for 24 h. Untreated cells and cells treated with only an effective dose PGD were served as controls. The medium and the cells were collected for the measurement of PRL secretion and synthesis, respectively.

GH3 and PC12 cells were treated with PGD at effective doses that had been examined in MMQ cells for 24 h. The medium and GH3 cells were collected for measuring PRL secretion and synthesis, respectively. PC12 cells were collected for detecting DAT and D_2 receptor expression.

2.3. In vivo experiment in animal model of hyperPRL

The experimental protocol was approved by Committee on the Use of Live Animals in Teaching and Research (CULATR) of LKS Faculty of Medicine of the University of Hong Kong. Sprague–Dawley female rats aged 9 weeks old and weighing 250–280 g were used in *in vivo* 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 experimental treatment started and blood samples were collected in the diestrus stage of the oestrous cycle.

To generate the experimental model of hyperPRL, 30 animals were given intraperitoneal (i.p.) metoclopramide (MCP, 150 mg/kg daily), a dopamine inhibitor for 10 days. This model has been widely used for investigation of hyperPRL (Laszczyńska et al., 2002). An additional group of 6 untreated animals was served as controls. At day 14, blood was drawn from the tail vein of both untreated and MCP-treated animals for measurement of PRL (see below). Since all 30 MCP-treated animals displayed an at least 80% elevation of serum PRL concentrations compared to an averaged level of untreated controls, all of them were used for experimental treatment and received gastric PGD at a dose of 0, 2.5, 5, or 10 g/kg, or i.p. injection of 0.6 mg/kg BMT for 14 days in a random manner ($n = 6$ per group). The selection of these doses was based on the dosages used in humans, as previously reported (Yuan et al., 2008). Blood samples were collected at the completion of experimental treatment and sera were separated for the measurement of PRL, progesterone, and estradiol.

2.4. Biochemical analyses

2.4.1. Hormone assay

PRL concentrations in the culture medium collected from MMQ and GH3 cells as well as PRL, progesterone, and estradiol concentrations in the rat sera were measured using enzyme-linked immunosorbent assay (ELISA) (Calbiotech, USA).

2.4.2. Western blotting

Western blotting was conducted to determine the expression of intracellular PRL in MMQ and GH3 cells as well as D_2 receptors and DAT in PC12 cells. Cell proteins were extracted and the concentration was determined using Bradford method as described previously. Proteins